University of São Paulo
"Luiz de Queiroz" College of Agriculture

Spatial distribution and temporal progress of *Huanglongbing* in areas with strict disease management

Kelly Pazolini

Thesis presented to obtain degree of Doctor in Science.
Area: Plant Pathology

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I dedicate with love to
my parents and sister Ivo, Ju e Bruna Pazolini,
my lovely grandparents Claudino e Onires
and my aunt Gemile

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"A viagem não acaba nunca. Só os viajantes acabam.

(...) O fim de uma viagem é apenas o começo de outra.

É preciso ver o que não foi visto, ver outra vez o que se viu já..."

José Saramago

CONTENTS

RESUMO

Distribuição espacial e progresso temporal de *Huanglongbing* em areas com manejo rigoroso da doença

A citricultura é uma importante atividade agroindustrial para o Brasil e grande parte da produção se concentra no estado de São Paulo. Entretanto, a produção de laranja enfrenta, atualmente, uma epidemia de *Huanglongbing* (HLB), doença considerada a principal ameaça à citricultura mundial. Apesar de muitos esforços no manejo, a incidência de HLB continua aumentando desde a sua detecção no estado de São Paulo, em 2004. A dificuldade de manejo de HLB está associada à migração do inseto vetor, *Diaphorina citri*, que adquire a bactéria em fontes de inóculo externas e ao efeito limitado do manejo rigoroso da doença realizado pelas fazendas (erradicação de plantas sintomáticas e controle frequente do vetor) sobre a disseminação primária da doença. Assim, estudos epidemiológicos podem facilitar o entendimento do complexo patossistema e auxiliar na decisão de medidas de controle mais eficientes. Os objetivos desse trabalho foram: 1) determinar o padrão espacial de HLB em 24 blocos cítricos sob o manejo rigoroso da doença e 2) estudar o progresso temporal da doença e a distribuição espacial de HLB e do vetor em uma propriedade de larga escala que realizou o manejo rigoroso da doença e sua associação com fontes externas de inóculo. Para verificar o padrão espacial de HLB, utilizou-se a analise função-K de Ripley modificada e mapas de densidade de kernel. Os resultados demonstraram que o padrão de distribuição regular de plantas sintomáticas de HLB prevaleceu nos blocos estudados e uma agregação fraca de aproximadamente 58 m foi observada nas bordas dos blocos. As maiores distâncias de agregação e médias de *D. citri*/armadilha ocorreram em blocos localizados na periferia das propriedades. O padrão espacial regular e a concentração da doença e do vetor na periferia da propriedade evidenciaram a importancia da disseminção primária na área. Para o estudo do progresso temporal, os modelos Gompertz e logístico foram ajustados ao progresso anual da doença de 177 blocos. A distribuição das taxas de progresso e incidência de HLB (para 177 blocos) e a média de *D. citri*/armadilha (para 296 blocos) foram visualizadas em mapas de gradiente. Os valores encontrados de taxa de progresso da doença, obtida com o modelo de Gompertz (de 0,04 a 0,28 por ano), incidência média de HLB (0,11) e média de *D. citri*/armadilha (0,016) foram baixos em comparação com dados da literatura. As variáveis apresentaram os maiores valores para blocos localizados na periferia da propriedade do que para blocos rodeados por citros. Uma grande quantidade de plantas não comerciais, e potencialmente servindo como fonte de inóculo, foi encontrada perto de blocos que apresentam as maiores taxas de progresso e incidências de HLB e médias de *D. citri*/armadilha. Os resultado encontrados demonstraram a importancia da disseminação primária em áreas que realizam o manejo rigoroso de HLB. Portanto, para reduzir novas infecções, o manejo do HLB precisa reduzir efetivamente as fontes externas de inóculo e o manejo regional deve incluir, além de pomares comerciais, plantas não comerciais.

Palavras-chave: Efeito de borda; Manejo regional; Fontes externas de inóculo; Plantas não comerciais

ABSTRACT

Spatial distribution and temporal progress of *Huanglongbing* in areas with strict disease management

Citriculture is an important agroindustrial activity for Brazil and citrus production is concentrated in São Paulo State. However, the activity is facing a *Huanglongbing* (HLB) epidemic which is considered to be the main threat to global citrus production. In spite of many management efforts, the incidence of HLB continues to increase since its detection in São Paulo State, in 2004. The difficulty of handling HLB is associated with the migration of the vector, *Diaphorina citri*, which acquires the bacteria in external inoculum sources and the limited effect of the strict HLB management performed by farms (eradication of symptomatic trees and frequent vector control) on the primary dissemination of the disease. Thus, epidemiological studies can facilitate the understanding of the complex pathosystem and help in the decision of more efficient control measures. The objectives of this study were: 1) to determine the spatial pattern of HLB in 24 citrus blocks under strict management of the disease and 2) to study the temporal progress of the disease and the spatial distribution of HLB and the vector in a large scale property that carried out the strict management of the disease and its association with external inoculum sources. To verify the spatial pattern of HLB, modified Ripley's K-function and kernel density estimation maps were used. The results showed that the regular distribution pattern of HLB symptomatic trees prevailed in the blocks studied and a weak aggregation of approximately 58 m was observed at the edges of the blocks. The largest aggregation distances and averages of *D. citri*/trap occurred in blocks located at the periphery of the properties.The regular spatial pattern and the concentration of disease and vector in the periphery of the properties evidenced the importance of primary dissemination. For the study of the disease temporal progress, the Gompertz and logistic models were adjusted to the annual progress of HLB for 177 blocks. The distribution of HLB progress rates and incidence (for 177 blocks) and average *D. citri*/trap (for 296 blocks) were visualized on gradient maps. The disease progress rate values obtained with the Gompertz model (0.04 to 0.28 per year), average HLB incidence (0.11) and average *D. citri*/trap (0.016) were low compared with literature data. The variables presented the highest values for blocks located at the periphery of the property, rather than for blocks surrounded by citrus. Many noncommercial plants, potentially serving as source of inoculum, were found near blocks with the highest rates of disease progress, HLB incidence and average *D. citri*/trap. The results demonstrated the importance of primary dissemination in areas that carry out a strict HLB management. Therefore, in order to reduce new infections, HLB management must effectively reduce external sources of inoculum and regional management should include, in addition to commercial orchards, noncommercial plants.

Keywords: Edge effect; Regional management; External inoculum soruces; Noncommercial plants

1. INTRODUCTION

Huanglongbing (HLB) is the most devastating disease of citrus and has spread to the main growing areas worldwide, including Brazil, USA and China. HLB is associated with three species of phloem-restricted proteobacterias, '*Candidatus* Liberibacter africanus', '*Ca*. L. asiaticus' and '*Ca*. L. americanus' (Gottwald, 2007). Two insect vectors are responsible for the fast spread of the disease: the Asian citrus psyllid (ACP), *Diaphorina citri*, in Asia and America, and the African citrus psyllid, *Trioza erytreae*, essentially in Africa (Duran-Vila & Bové, 2015). *D. citri* has been associated with citrus in Brazil since 1942 (Costa Lima, 1942), while the detection of '*Ca*. L. spp.' occurred in 2004 in the central area of São Paulo State (Coletta Filho *et al*., 2004; Teixeira *et al*., 2005).

HLB epidemics has impacted citrus cultivation from individual farms to national economies, therefore impacting food security (Bergamin Filho *et al*., 2016) by declining fruits production and quality (Gottwald *et al*., 2010). Furthermore, the disease is responsible for shortening the life span of orchards (Das, 2004) and increasing productions cost, often leading to economic unfeasibility of citrus activity. In Brazil, it was estimated that more than 40 million HLB symptomatic trees had been removed by 2016 (CDA, 2017) and that more than 35 million orange trees (18.15%) are currently HLB symptomatic in the Citrus Belt region, main orange juice producer region in Brazil (Fundecitrus, 2018).

There are no viable curative strategies or resistant citrus variety to control HLB yet, and disease management is based in the reduction of inoculum, i.e., utilization of healthy nursery trees, eradication of symptomatic trees, and chemical control of vector populations (Belasque *et al*., 2009). However, even in groves applying this management, HLB can cause infection in 100% of trees in 2 to 5 years (Bassanezi *et al*., 2013; Belasque *et al*., 2010). The low efficiency managing HLB has being related to the disease primary spread. While the secondary spread, i.e., the transmission of the pathogen to trees on the same orchard, can be reduced by insecticide sprays, the primary spread, i.e., the pathogen entry in property edges by the vector migration, is very difficult to control and, therefore, is responsible for introducing the disease even in areas that perform rigorous management. According to Gottwald *et al*. (2010), the primary spread is the most dangerous kind of spread as it indicates a spatial process of long distance, and then explain why control efforts on a local scale have always failed.

Taking into account the importance of HLB to national economy and the difficult in reducing disease incidences, epidemiology can be an important tool to gain valuable information and improve HLB management strategies. Detailed information on the spatio-temporal dynamics of plant diseases, i.e., estimation of the key epidemiological parameters, enables a more complete picture of

the structure and behavior of the pathosystem. This information can be used to predict the likelihood and extent of further spread, to describe and understand the development of diseases, developing sampling plans, planning controlled experiments, to characterize losses caused by the disease and to quantify the effectiveness of different strategies for disease control (Bergamin Filho *et al*., 2002; Parry *et al*., 2014).

Therefore, the objectives of these studies were to characterize the spatial and temporal progress of HLB in properties with a strict disease management in São Paulo State. These analyses in Brazilian conditions are scarce and can bring important information regard to HLB epidemic characterization. Furthermore, these findings can help to analyze current HLB control program as well to improve disease management strategies.

REFERENCES

Bassanezi RB, Montesino LH, Gimenes-Fernandes N *et al.*, 2013. Efficacy of area-wide inoculum reduction and vector control on temporal progress of *Huanglongbing* in young sweet orange plantings.*Plant Disease* **97**, 789–796.

Belasque J, Bassanezi RB, Yamamoto PT *et al.*, 2010.Lessons from *Huanglongbing* management in São Paulo State, Brazil. *Journal of Plant Pathology* **92**, 285–302.

Belasque J, Bergamin Filho A, Bassanezi RB *et al.*, 2009. Base científica para a erradicação de plantas sintomáticas e assintomáticas de *Huanglongbing* (HLB, Greening) visando o controle efetivo da doença. *Tropical Plant Pathology* **34**, 137–145.

Bergamin Filho A, Hau B, Amorim L*et al.,* 2002. *Revisão Anual de Patologia de Plantas: Análise espacial de epidemias*. EMBRAPA **10**, 155–218.

Bergamin Filho A, Inoue-nagata AK, Bassanezi RB *et al,* 2016*.* The importance of primary inoculum and area-wide disease management to crop health and food security. *Food Security* **8**, 221–238.

CDA (Defesa Agropecuaria do Estado de São Paulo), 2017. Compilação dos dados dos relatorios semestrais do greening enviados para a coordenadoria de Defesa Agropecuaria do Estado de São Paulo (CDA). *Dados da Citricultura Paulista*.

Coletta Filho HD, Targon MLPN, Takita MA *et al.,* 2004. First report of the causal agent of *Huanglongbing* ('*Candidatus*Liberibacterasiaticus') in Brazil. *Plant Disease* **88**, 1382.

Costa Lima AM, 1942. *Insetos do Brasil, Homoptera.* Escola Nacional de Agronomia. Série Didática.

DAS AK, 2004. Rapid detection of '*Candidatus* Liberibacter asiaticus', the bacterium associated with citrus *Huanglongbing* (Greening) disease using PCR. *Current Science***87**, 1183–1185.

Duran-Vila N, Bové JM, 2015.Citrus HLB is an emerging disease transmitted by psyllid vectors. Can it be prevented? If not, can it be managed? *Watch Letter* **33**.

Fundecitrus, 2018. Levantamento da incidência das doenças dos citros: Greening, CVC e Cancro cítrico no cinturão citrícola de São Paulo e triângulo/sudoeste mineiro. *Fundo de Defesa da Citricultura – FUNDECITRUS*.

Available in: https://www.fundecitrus.com.br/levantamentos/greening

Gottwald TR, 2007. Citrus canker and citrus *Huanglongbing*, two exotic bacterial diseases threatening the citrus industries of the Western Hemisphere. *Outlooks on Pest Management* **18**, 274–279.

Gottwald TR, 2010. Current epidemiological understanding of citrus *Huanglongbing*. *Annu. Rev. Plant Phytopathol* **48**, 119–139.

Gottwald TR, Irey M, Gast T *et al.,* 2010. Spatio-temporal analysis of an HLB epidemic in Florida and implications for spread. *Proceedings of the 17 th Conference of International Organization of Citrus Virologists, IOCV*, 84–97.

Parry M, Gibson GJ, Parnell S *et al.*, 2014. Bayesian inference for an emerging arboreal epidemic in the presence of control. *Proceedings of the National Academy of Sciences of the United States of America* **111**, 6258–62.

Teixeira DDC, Danet JL, Eveillard S *et al.*, 2005. Citrus *Huanglongbing* in São Paulo State, Brazil: PCR detection of the '*Candidatus* Liberibacter' species associated with the disease. *Molecular and Cellular Probes* **19**, 173–179.

2. LITERATURE REVIEW

2.1 Importance of HLB

Huanglongbing (HLB) is considered the most devastating disease of citrus and is present in many producing regions worldwide, including the three major citrus-producing countries: Brazil, the USA and China (Bové, 2014). The effects of HLB have impacted citrus production from individual farms to national economies by quickly declining fruits production and quality (Gottwald, 2010; Farnsworth *et al*., 2014), shortening the life span of orchards (Das, 2004) and increasing production costs. The citrus activity become unprofitable for many small and medium producers, leading to abandonment of orchards and causing a thousand of direct and indirect unemployment (Hodges & Spreen, 2012). Furthermore, there is no commercial variety of canopy or rootstock resistant to HLB and infected plants cannot be cured, yet. All these factors have made the citrus production a challenge.

According to da Graça *et al*. (2016) and Bergamin Filho *et al*. (2016), HLB is a complex disease that involves interactions among the pathogen, vector, hosts and the environment in its broadest definition. Moreover, the disease also account with a long incubation period, the inability, until now, to culture the bacteria, and the lack of any known sources of natural resistance (da Graça *et al*., 2016). These factors can explain why the disease has being causing so many losses since its detection, almost 100 year ago, and is still epidemic.

HLB was first reported in China, in 1919, but most likely originated in Taiwan in the 1870s (Bové, 2006; Lin, 1956). It was estimated that more than 100 million infected citrus trees have been destroyed by the disease throughout Asia (Zhang *et al.*, 2014). USA and Brazil, two relatively new areas where the disease was detected, had the pathogen first report in 2004 in the central area of São Paulo State (Coletta Filho *et al*., 2004; Teixeira *et al*., 2005) and in 2005 in South Florida.

In Florida, 80% of orange trees were estimated to be HLB-infected (Singerman & Useche, 2016). Since the disease detection, HLB has reduced citrus area and yields by 26% and 42%, respectively, causing a devastating impact on industry (Institute of Food and Agricultural Sciences, 2016). According to Spreen & Baldwin (2013), HLB had caused an estimated loss of $1.3 billion in direct revenue and $3.6 billion in indirect revenue until 2013. In Brazil, it was estimated that more than 40 million trees had been removed by 2016 (CDA, 2017). It was also recently estimated that 35.3 million orange trees (18.15%) are currently HLB symptomatic in the Citrus Belt region, main orange juice producer region in Brazil (Fundecitrus, 2018a).

Citrus Belt region is composed of São Paulo and west-southwest of Minas Gerais States. According to the inventory carried out by Fundecitrus in 2018 (Fundecitrus, 2018a), Citrus Belt region is composed by 5,882 orange growing properties, of which 64% are composed of less than 10,000 trees. Related to cultivated varieties, nearly 90% of the Citrus Belt is formed by five oranges: Pera Rio (35%), Valencia (27%), Hamlin (11%), Natal (11%) and Valencia Folha Murcha (4%). In the same region, the citrus planted area showed a decrease of 43,933 ha (7%) in 2018 comparing with 2015.

The reduction of citrus area in Citrus Belt is related, in part, to the abandonment of the activity mainly by small and medium producers. Low citrus prices, the high costs in the management of HLB and the fast reduction of productivity of infected orchards made the activity economically unfeasible and forces many producers to abandon the activity. According to the inventory, there are currently 6,050 ha of abandoned orchards in Citrus Belt. In Florida, this number was estimated to be 52,886 ha in 2016 (USDA, 2017). Cumming &George (2009) related the number of abandoned groves in Florida with socioeconomic pressures, freezing events, and the loss of production because of canker and HLB diseases.

Abandoned areas or areas with infected trees and no HLB management become inoculum sources and puts at risk the properties in which the strategies of HLB control are effectively employed (Bassanezi *et al*., 2013; Spreen & Baldwin, 2013). It is known that the vector *D. citri* is able to disperse several kilometers without wind assistance (Martini *et al.*, 2013, 2014; Lewis-Rosenblum *et al.*, 2015) and move frequently from abandoned to managed orchards (Boina *et al.*, 2009; Tiwari *et al.*, 2010; Lewis-Rosenblum *et al.*, 2015). The movement of an infective *D. citri* from an orchard where it acquired the pathogen to another orchard is called primary spread.

Besides abandoned areas or areas with no HLB management, the maintenance of infected trees on sidewalks and backyards can be an even bigger problem to HLB management. These plants do not receive any chemical treatment for vector control and are often unnoticed as important sources of inoculum. Because of the importance of these trees, Fundecitrus, a citrus growers association, launched, in 2017, the “United Against Greening” campaign to raise awareness of the society on the impact of HLB and the need of inoculum sources removal (Fundecitrus, 2018b).

In the search for ways to reduce the devastating effects of HLB in citriculture, many changes are and still will be made so that the production of citrus keeps viable. According to da Graça *et al*. (2016), keep an economic production of citrus is the largest challenge ever faced by the citrus industry worldwide. The current scenario involves a lot of resources investment by government and citrus industry in researches focusing in finding and implementing strategies to minimize the infections and the symptoms progress on citrus trees.

2.2 HLB etiology and symptomatology

HLB is associated with three species of phloem-restricted proteobacterias, '*Candidatus* Liberibacter africanus' (Claf), '*Ca*. L. asiaticus' (Clas) and '*Ca*. L. americanus' (Clam) (Gottwald, 2007). In Brazil, two Liberibacter species are associated with the disease, Clas and Clam. Although genetically closely related, Clas tolerates higher temperatures, is more adapted to citrus and more efficiently transmitted by psyllid than Clam (Lopes *et al*., 2009a,b; da Graça *et al*., 2016). Since the detection of Clam in Brazil, in 2005, its occurrence decreased from 98 to 20% in a period of four years, and Clas is currently predominant (da Graça *et al*., 2016).

HLB bacterium have not been maintained in sustained pure culture until now (Davis *et al.*, 2008) and its characterization was achieved around 1990s with DNA-based techniques (PCR and 16SrDNA). The pathogen was, then, confirmed to be Gram-negative bacteria belonging to a new genus '*Candidatus* Liberibacter' in the alpha subdivision of the Proteobacteria (Bové, 2014).

The three species of *'Candidatus Liberibacter'* are transmitted from plant to plant by grafting, psyllid vectors or experimentally by dodder. Two species of citrus psyllids are associated with HLB transmission: the Asian citrus psyllid (ACP), *Diaphorina citri*, in Asia and America, and the African citrus psyllid, *Trioza erytreae*, essentially in Africa (Duran-Vila & Bové, 2015). The ACP is a phloem specialist vector that acquires and transmits the bacteria while feeding on flush shoots of rutaceous host plants. Therefore, psyllids are essential to natural spread of the disease.

All known citrus varieties are susceptible to HLB (Folimonova *et al.*, 2009). The ornamental rutaceous *Murraya paniculata* (orange jasmine) also has been confirmed as a host for both, Clam and Clas, in Brazil (Lopes *et al*., 2005, 2006). The bacteria were confirmed to multiply in psyllids and in the hosts (Lee *et al.*, 2015).

After transmitted, the pathogens are carried through the flow of the sap to the entire plant, blocking the transport of sucrose to the root system and causing starch accumulation in the foliage, deterioration of the root system and nutrients deficiency symptoms (Kim *et al.*, 2009; Etxeberria *et al.*, 2009). The highest concentrations of Clas are found in the stem and midribs of flush (Lee *et al.*, 2015). HLB symptoms can be seen in any period of the year but are more often between early spring and late summer. Initial HLB symptoms include yellow branches sparsely foliated with mottled leaves and small lopsided fruits. Infected branches produce small, misshapen and bitter fruits. According to Bassanezi *et al*. (2017), infected fruits have lower percentage of juice and juice can be 5 to 45% more acidic. HLB symptomatology evolves to severe defoliation, fruit drop and death of the branches (Bové, 2006; da Graça *et al*., 2016). Tree death may occur as result of defense gene expression in the plants, which cause the blockage of the phloem (Kim *et al.*, 2009; Folimonova *et al.*, 2009).

The latency period, i.e., the time between infection and the time that the pathogen is accessible to another vector acquisition, can be as short as 15 days (Lee *et al.*, 2015). Unlike many other diseases, the latent and the incubation periods (onset of symptoms) can be vastly dissimilar (Boina & Bloomquist, 2015). While the latent period is as short as a single generation of psyllids (15 days), the incubation period can stretch from months to 6 years (Shen *et al*., 2013). The latent period depend on the age and nutritional status of the infected tree, bacterial and citrus species and climate conditions (Bové, 2006; Gottwald, 2010b; Shen *et al*., 2013). Consequently, trees infected at the same time may express the symptoms in different periods.

The incubation period of HLB is a limiting factor for the eradication practice. In addition to requiring trained staff for plant inspections, because the symptoms are very similar with symptoms of nutritional deficiency, inspections are useful only for symptomatic plants. Thus, while symptomatic trees are frequently removed in managed orchards, asymptomatic trees may be acting as inoculum sources.

2.3 *Diaphorina citri*

The vector Asian citrus psyllid (ACP) *Diaphorina citri* (Kuwayama) (Hemiptera: Liviidae) has been associated with citrus in Brazil since 1942 (Costa Lima, 1942), while both Clas and Clam were first described in 2004 in São Paulo State (Coletta Filho *et al*., 2004; Teixeira *et al*., 2005). Before detection of HLB, *D. citri* was considered a secondary pest. However, due to its ability to transmit HLB, the ACP is currently one of the most important vectors of world citriculture(Costa *et al*., 2010). In Florida, *D. citri* was first detected in 1998 (Halbert, 1998), but the psyllid quickly established in citrus producing regions and, by September 2000, had spread to 31 Florida counties (Halbert *et al*., 2001).

Development of ACP occurs in the presence of young citrus leaves where the female places its eggs and nymphs feed. Adults *D. citri* are able to feed on young and mature leaves, whereas early instars of *D. citri* feed only on flush shoots of rutaceous host plants (Hall & Albrigo, 2007; George *et al*., 2017). Thus, *D. citri* densities are strongly related with presence of new flush shoots (Yasuda *et al.*, 2005; Tomaseto *et al.*, 2015; Sétamou *et al.*, 2016). The predilection of *D. citri* for young leaves is related, probably, to the higher concentrations of macro and micro nutrients and the ease of probing by the insect's mouthparts (Sétamou *et al.*, 2016; George *et al.*, 2017).

Temperatures between 24°C and 30°C are the most favorable for insect survival and reproduction and, under this condition, adults life cycle lasts 30 to 50 days and females laid 500 to

800 eggs (Tsai & Liu, 2000; Nava *et al.*, 2007; Hall *et al.*, 2011). However, there are studies showing that *D. citri* can survive from 21 to 117 days (Liu & Tsai, 2000; Nava *et al*., 2007).

Both *D. citri* nymphs and adults can acquire the HLB pathogen when feeding on infected plants (Capoor *et al*., 1974; Xu *et al*., 1988), but it's a poor vector when the pathogen is acquired during the adult stage, unlike adults emerged from nymphs that acquired the pathogen (Inoue *et al.*, 2009; Pelz-Stelinski *et al.*, 2010). It takes 5 to 7 hours of feeding to *D. citri* acquire the pathogen (Halbert & Manjunath, 2004) and only 40% of adults insects successfully acquire the bacteria (Pelz-Stelinski *et al.*, 2010). Also, a latent period of 1 to 25 days may be required before transmission of HLB when it is acquired by *D. citri* adults (Huang *et al*., 1984; Pelz-Stelinski *et al.*, 2010; Grafton-Cardwell *et al.*, 2013). On the other hand, when acquired at the nymphal stage, adults are able to transmit HLB immediately after emergence (Inoue *et al*., 2009; Capoor *et al*., 1974; Xu *et al*., 1988). Clas is transmitted by *D. citri* in a persistent and propagative manner. According to Ammar *et al*. (2016), Clas appears to multiply in both, nymphs and adults, but adults may require longer time feeding on infected plants for Clas to reach higher levels in the vector.

Although nymphs are more efficient in acquiring and transmitting HLB, the adults play a more important role as they are responsible for disease spread. Its known that *D. citri* is able to disperse many kilometers without wind assistance (Martini *et al.*, 2013, 2014; Lewis-Rosenblum *et al.*, 2015) and, therefore, may disseminate HLB within and between groves (Halbert & Manjunath, 2004). According to Kobori *et al*. (2011), adults initiate dispersion 3 to 4 days after emerging.

Boina *et al*. (2009) verified that *D. citri* might move at least 100 m between groves in 3 days period and Lewis-Rosenblum *et al*. (2015) verified that the psyllids were able to disperse at least 2 km within 12 days. Also, geographical barriers are known to have little impact on the vector dispersal as the psyllid has been captured in a dense forest 2.3 km away from a citrus grove (Martini *et al.*, 2013) and in urban environments (Chong *et al.*, 2010; Godfrey *et al.*, 2013).

Therefore, due *D. citri* abilities to disperse to long distances, abandoned citrus areas or unmanaged hosts play an important role as a reservoir for HLB (Boina *et al.*, 2009; Tiwari *et al.*, 2010; Lewis-Rosenblum *et al.*, 2015). Furthermore, as studied by Tiwari *et al*. (2010), psyllids tended to migrate from abandoned plantings into managed groves. For these reasons, vector control and trees rouging must be performed in a regional scale (Bassanezi *et al*., 2013).

2.4 Management of HLB in Brazil

As already mentioned, there are no citrus commercial varieties of canopy or rootstock resistant to HLB and infected plants cannot be cured yet. According to da Graça *et al*. (2016), the lack of HLB natural resistance in citrus is one of the major factors contributing to the rapid spread and devastation of the disease. For now, integrated management is the most efficient way to reduce HLB effects and must be done simultaneously by all citrus growers in a regional scale to reduce primary spread effects (Bassanezi *et al*., 2013; Bergamin Filho *et al*., 2016). Since 2006, and based on other countries experience, three basic HLB management strategies have been indicated and adopted, at different levels, in São Paulo citrus properties: use of healthy nursery trees, inspections and eradication of symptomatic trees and chemical control of the vector, *D. citri*.

Contaminated citrus seedlings can be an important mean of spreading HLB, as the commercialization can occur at long distances and may introduce HLB into areas still free of the disease. Also, sooner the trees get infected, shorter will be the productive life of the orchard. Therefore, it is essential to acquire healthy nursery trees for formation of new orchards or to replanting eradicated trees.

The use of certified citrus seedling in São Paulo State was implemented after a Citrus Variegated Chlorosis (CVC, *Xylella fastidiosa*) epidemic, in 1990. Transmission of CVC by spittlebugs led the Department of Agriculture and Food Supply of the State of São Paulo to create a public normative, from 2000, regulating citrus propagating material production. The normative induced significant changes such as mandatory production of seedlings in insect-proof green houses and high standards of sanitation (CDA, 2017). With the implementation of these phytosanitary norms, the risk of introducing diseases in citrus orchards through seedlings was significantly reduced. When HLB was first reported (2004), São Paulo industry was already producing and planting certified nurseries seedlings. Thus, the HLB spread in Brazil is predominantly associated with the movement of *D. citri*.

Florida initiated the implementation of phytosanitary norms to production of seedlings in an ACP-free environment only in 2008 (Halbert *et al.*, 2012), after HLB introduction, in 2005. The delay in seedlings certification is one of the main causes of the faster spread of the disease in the USA. While in Brazil it was estimated that HLB expanded, approximately, 20 km per year (Gottwald *et al*., 2007), in Florida, the estimation was 50-60 km per year (Halbert *et al*., 2008).

Regard to the eradication of symptomatic trees, frequent inspection of all trees is an important strategy aiming the early detection of HLB. Surveys should be performed by well-trained staffs, as disease initial symptoms can be easily confused with symptoms of nutritional deficiency. In orchards with adult trees, platforms of four inspectors (two observing the top and two observing the

skirt of the trees) are indicated (Belasque *et al*., 2009). However, even in such conditions, according to Bové (2014), only 60% to 70% of symptomatic trees are detected. Besides that, inspections of all trees by trained staffs are expensive and take a long time.

Even if a 100% of symptomatic trees were detected by visual inspections, and eradicated, it wouldn't mean the total elimination of infected trees (inoculum sources), as HLB incubation period is variable. In this sense, techniques for identification of asymptomatic trees would be of great help so HLB infected trees could be eradicated as soon as possible. An early and fast diagnosis of '*Ca.* Liberibacter' is critical in reducing the spread and devastation of HLB, as well as minimizing the economic impact of potential false-positive diagnoses (Valdés *et al*., 2016). Many efforts have been made for development of a fast and viable technique for HLB detection, such as real-time PCR (Orce *et al.*, 2015), visible spectrum image (Deng *et al.*, 2016), optical sensor (Mishra *et al.*, 2011), among others.

Orange jasmine (*Murraya paniculata*; Rutaceae), an ornamental plant frequently used in Brazil and alternative host to *D. citri* and HLB bacteria, also need to bea target on a program of eradication and ACP control. This plant is an inoculum source in HLB epidemics and its importance shouldn't be underestimated.

Among the three basic strategies for HLB management, eradication of symptomatic trees is the most controversial and difficult to accomplish by citrus growers (Martini *et al.*, 2015). This activity is expensive as involves frequent symptom inspections, eradication of the symptomatic trees, direct impact on production by eradication of productive trees and costs of seedlings replanting (Parnell *et al.*, 2010; Bassanezi *et al.*, 2013b).

Different from trees eradication, the chemical control of *D. citri* is better accepted and carried out by most of the producers. The effective monitoring and control of the psyllid are essential for HLB management. The most frequent used method of monitoring ACP in Brazil are the yellow sticky cards (traps), but visual inspection of young citrus shoots is also used to detect the presence of eggs, nymphs and adults (Belasque *et al.*, 2010). The greater detection of *D. citri* nymphs in branches may indicate a bad chemical management, as the developmental period from egg to adult may be as short as 14 days (Liu & Tsai, 2000).

Monitoring the vector population is an important activity for the decision of insecticide applications (Bassanezi *et al*., 2013), and so, at least one trap per citrus block or a regular distribution of traps is indicated in the property. Also, periods of the highest vegetative activity of the crop, when control measures may be more efficient due to the lower insect activity, must be taken into account (Tomaseto *et al.*, 2015). Flushes at emerging and developmental phases offer optimal conditions for feeding and oviposition of *D. citri* and are attractive by a combination of chemical volatiles and visual stimuli (Patt *et al*., 2014; Patt & Sétamou, 2010; Wenninger *et al*., 2009).

Usually, *D. citri* detection (Sétamou & Bartels, 2015) and HLB incidences (Bassanezi *et al*., 2013; Bergamin Filho *et al*., 2016; Gasparoto *et al*., 2018; Monteiro *et al*., 2013) are concentrated on the border of the properties (border effect). Thus, many citrus growers intensify the management in the border of the citrus blocks, by increasing the frequency of insecticide applications in these areas (Miranda *et al.*, 2018). Besides contact insecticide sprays, systemic insecticide treatment of young orchards, until three year old, is highly indicated due the higher vegetative activity in the trees. (Bové, 2014; Rogers & Shawer, 2007; Sétamou *et al*., 2010).

However, even a property carrying a very strict management of *D. citri,* with use of healthy seedling, frequent inspections and HLB-tree eradication and frequent insecticide sprays, it won't achieve total prevention of primary infections due insect's ability to dispersal among citrus groves (Halbert & Manjunath, 2004; Boina *et al.*, 2009; Martini *et al.*, 2013, 2014; Lewis-Rosenblum *et al.*, 2015). The high rates of HLB spread and external inoculum sources are the main difficulties of controlling the disease (Bové, 2014; Gottwald *et al*., 2010b).

Therefore, for successful HLB management, the removal of symptomatic trees and psyllid control must be accomplished regionally by citrus growers, and include residential citrus and abandoned orchards (Bassanezi *et al*., 2013; Bergamin-Filho *et al*., 2016). However, an area-wide management is not simple to accomplish due the difficulty for coordinating control actions among neighboring farmers and government regulation is required (Bergamin Filho *et al*., 2016; Tomaseto *et al*., 2017). Seeking to facilitate an area-wide coordinate management, programs developed by Fundecitrus, such as the “Phytosanitary Warning System” and the "United Against Greening" campaign have been assisting citrus growers.

The “Phytosanitary Warning System” organizes information of the regional monitoring of *D. citri* populations by georeferenced traps and vegetative stage of citrus plants. The program alerts producers of critical moments for regional control of *D. citri*. The "United Against Greening" campaign aims to raise awareness of the society on the impact of HLB and the need of removing hosts plants on sidewalks or backyards (Fundecitrus, 2018b). These plants do not receive any chemical treatment for ACP control and are often unnoticed as important sources of inoculum.

An important alternative control method of *D. citri* that had been widely used in the regional management is the ectoparasitoid biocontrol agent *Tamarixia radiata* (Waterston, 1922) (Hymenoptera: Eulophidae). A single ectoparasitoid is able to parasites up to 500 nymphs (Skelley & Hoy, 2004) with a parasitism rate of more than 77% (Gómez-Torres *et al.*, 2012). The parasite is very useful on regional management as the insects can be released on areas with inoculum sources that are unknown, such as woods, or areas where eradication and sprays with insecticides are not allowed, such as backyards.

Some other important aspects to the management of HLB are the size and shape of the properties, age of the orchard, incidence of HLB in the area where the property is located and presence of neighboring properties with no HLB management. Due the border effect, small or narrow orchards will be more affected due the greater periphery area than large and square farms (Bové, 2014). Many times, citrus production may lose economic viability to small producers, what can lead to a dangerous cycle: abandoned properties became HLB inoculum sources to others properties (Bové, 2014).

With the lack of resistant cultivars and curative methods, HLB epidemics can be relatively fast and destructive for local citrus industries if no effective control measures are undertaken immediately (Bassanezi & Gottwald, 2009). However, to keep citrus orchards producing without contracting or with low HLB incidence is costly and may be difficult even under intensive management programs. The current scenario involves a lot of resources investment by government and citrus industry in researches focusing in finding and implementing strategies to minimize the infection and the symptoms progress on citrus trees.

2.5 Epidemiology of HLB

Although the high and increasing HLB incidences and the concerning situation of the agribusiness chain of citrus, relatively few quantitative epidemiological studies have been conducted in Brazil. Epidemiology is an important tool to predict the likelihood and extent of further spread, to describe and understand the development of diseases, developing sampling plans, planning controlled experiments, to characterize losses caused by the disease and to quantify the effectiveness of different strategies for disease control (Bergamin Filho *et al*., 2002; Gottwald *et al*., 2010a; Parry *et al*., 2014).

However, studying HLB epidemiology can be a difficult task due, mainly, the variable lag on the HLB expression of the symptoms, the perennial nature of the disease and host and the difficulty to locating study sites where the disease is allowed to progress without intervention of control activities (Bassanezi *et al*., 2010; Gottwald *et al*., 2010a). Although accuracy of temporal and spatial studies can be affected by these factors, useful information can still be obtained (Gottwald *et al.*, 2007; Gottwald, 2010b).

Temporal dynamics of HLB were investigated, initially, in Reunion Island and Southern China (Gottwald, 1989; Gottwald *et al*., 2007). Gottwald *et al*. (2010b), using logistic and Gompertz linear models to explain HLB epidemic in Florida, obtained rates varying from 0.72 to 2.73 for the

logistic model and 0.43 to 0.97 for Gompertz model. Bassanezi *et al*. (2013a) and Bassanezi *et al*. (2013b), obtained HLB rates ranging from 0.82 to 2.77 through linear regression of the logistic model in citrus blocks under strict management in Brazil. Arruda (2017) found logistic rates ranging from 0.28 to 1.88 and Gompertz rates ranging from 0.13 to 1.00, using non-linear regression, on data of HLB epidemic progress in properties under strict HLB management in São Paulo. According to Bassanezi and Gottwald (2009), taking into account the perennial nature of citrus plantings, HLB epidemics can be considered a fast disease. In absence of management in young plantings, disease incidence can reach more than 50% of the trees in 3 to 5 years.

Analysis of spatial distribution of infected trees had also brought important information in the understanding of the HLB pathosystem. According to Taylor (1984), the knowledge of the spatial distribution of a disease provides valuable references to the main factors that explain its nature. These analyses are essential for understanding the behavior of an infection between sick plants at near and long distances, as well as the influence of the vector in both processes (Gottwald *et al.,* 2007). According to Shen *et al*. (2013), understanding spatial patterns of HLB spread may help identify control measures to reduce the spread of disease and address questions on where the greatest risk is and how it is likely to be reduced in certain locations.

A characteristic of the spatial distribution of HLB is the edge effect, i.e., disease incidences are characteristically higher on the border of properties. The edge effect is caused by the migration of infective psyllids from nearby areas without or poor HLB management and, as a consequence of *D. citri* migration, most of the symptomatic trees can be seen on the peripheral areas of the properties. Therefore, the border effect is an indication of psyllid migration (primary spread). This characteristic in the spatial distribution of plants infected with HLB has already been observed and analyzed by several researchers (Bassanezi *et al*., 2005, 2006; Gottwald *et al.,* 2008; Gottwald, 2010b; Gottwald *et al*., 2007; Sétamou, 2014; Sétamou & Bartels, 2015; Shen *et al*., 2013; Tomaseto *et al*., 2015). Gottwald *et al*. (2008) also point out that besides the edges, infections caused by HLB tend to occur in areas of orchards intersected by roads, canals, containers, houses, buildings and equipment.

Gottwald *et al*. (2010a), using spatio-temporal Markov-Chain Monte Carlo (MCMC) analysis to study the spread of HLB in citrus blocks in south Florida, verified that spatial progress of HLB can occur due to only primary or secondary dissemination, but, mostly, it occurs as an incessant mixture of both spatial processes. Despite both processes importance to HLB epidemic, secondary spread is easily reduced by insecticide sprays, but the pathogen entry in property edges, carried by vectors, is very difficult to control. Thus, even properties performing intensive HLB management are constantly re-infested and subject to continuous primary infections, especially in the borders of the groves (Bergamin Filho *et al*., 2016), and a regional management is necessary (Bassanezi *et al.*, 2013b; Bergamin Filho *et al.*, 2016; Gasparoto *et al.*, 2018).

The presence of properties without or with poor HLB management have been a major problem for efficient HLB management (Bové, 2014). According to Gottwald *et al*. (2010a), the primary dissemination is the most dangerous kind of spread because it indicates a spatial process of long distance or regional spread, and then, it explains why control efforts on a local scale have always failed. This was later confirmed by Bassanezi *et al*. (2013), which demonstrated that the control effects of the primary dissemination is only possible with an area-wide inoculum reduction and vector control.

The spatial dynamics of HLB was investigated in many places, as the Reunion Island, China, Philippines (Gottwald *et al*., 1989; Gottwald *et al*., 1991; Gottwald *et al*., 1991), Brazil (Bassanezi *et al.*, 2005, 2006; Leal *et al.*, 2010), Cuba (Batista *et al*., 2013), the USA (Gottwald *et al*., 2008; Gottwald *et al.*, 2008b,a; Gottwald, 2010; Shen *et al.*, 2013b; Sétamou *et al*., 2014) and Mexico (Pèrez *et al*. 2016). In all studies areas, it was observed aggregation of the HLB diseased plants, except when the incidence was extremely low or high. In a study performed in Brazil, aggregation within quadrats was not observed in the majority of blocks when disease incidence was low, indicating that infective vectors land at random in a field at the beginning of the epidemic, before they become established in a tree (Bassanezi *et al.*, 2005).

Evidences of HLB transmission among adjacent trees were demonstrated by the ordinary runs analysis. However, the percentage of aggregation within and across rows was low, being the within row aggregation slightly stronger than across-row aggregation (Gottwald *et al.,* 1991; Bassanezi *et al.*, 2005; Shen *et al.*, 2013b). Therefore, vector movement appears to occur both from one tree to those within the immediate vicinity, and over a larger scale to trees at 25 to 50 m distance, the latter initiating new foci of infection (Gottwald *et al*., 1989; Gottwald *et al*., 1991; Bassanezi *et al.*, 2005).

Gottwald *et al*. (2010b) used modified Ripley's K-function method to present the first regional examination of spatial distribution of HLB. Data was obtained from a 4,800 ha plantation in South Florida where infection was entirely dependent on psyllid transmission. Modified Ripley's K-function analyses demonstrated a continuous relationship between HLB diseased trees in a range up to 3.5 km. A range from 0.88 to 1.61 km was also indicated as the most common distance between pairs of diseased trees, which may indicate a mean distance of psyllid regional dispersion. Modified Ripley's K-function describes the interaction or spatial dependence between points through space (Ripley, 1981). The analysis pairwise distances between diseased trees to assess clustering versus regularity and has been widely used in the study of plant diseases, including citrus canker (Gottwald *et al*., 2002) and citrus black spot (Spósito *et al.*, 2007; Hendricks *et al.*, 2017).

Epidemiological analyses of HLB in Brazil, such as modified Ripley's K-function, are scarce. However, this information is important for understanding the epidemic in Brazilian conditions and to improve the disease control.

REFERENCES

Ammar el-D, Ramos JE, Hall DG *et al.,* 2016. Acquisition, replication and inoculation of '*Candidatus* Liberibacter asiaticus' following various acquisition periods on *Huanglongbing*-infected citrus by nymphs and adults of the Asian citrus psyllid. *Plos One* **11**, e0159594.

Arruda JH, 2017. Disseminação primária e influência de fontes de inóculo sobre o progresso temporal do *Huanglongbing* em pomares com manejo da doença e seu vetor (*Diaphorina citri*). Tese de doutorado. Esalq.

Bassanezi RB, Gottwald TR, 2009. Epidemiology of HLB and potencial pathways for introduction. In: *Internacional Worckshop on citrus quarantine pest*.

Bassanezi RB, Miranda MP, Lopes AS *et al,* 2017. Influência de pragas e doenças na qualidade do suco de laranja. *Revista Citricultor*.

Bassanezi RB, Belasque J, Montesino LH, 2013a. Frequency of symptomatic trees removal in small citrus blocks on citrus *Huanglongbing* epidemics. *Crop Protection* **52**, 72–77.

Bassanezi RB, Bergamin Filho A, Amorim L *et al.,* 2006. Epidemiology of *Huanglongbing* in São Paulo. *Huanglongbing-Greening International Workshop Proceeding* **37**.

Bassanezi RB, Lopes S, Belasque J *et al.,* 2010. *Huanglongbing* epidemiology and implications of disease managementt. *Citrus Research & Technology,* **31**, 11–23.

Bassanezi RB, Busato LA, Bergamin Filho A *et al.,* 2005. Preliminary spatial pattern analysis of *Huanglongbing* in São Paulo, Brazil. *Sixteenth IOCV Conference* **16**.

Bassanezi RB, Montesino LH, Gimenes-Fernandes N *et al.*, 2013b. Efficacy of area-wide inoculum reduction and vector control on temporal progress of *Huanglongbing* in young sweet orange plantings. *Plant Disease* **97**, 789–796.

Batista L, Hernández L, Luis M *et al.,* 2013. Estudios epidemiológicos de la enfermedad *Huanglongbing* de los cítricos en tres localidades de cuba. *Instituto de investigaciones en fruticultura tropical* **78**.

Belasque J, Yamamoto PT, Miranda MP *et al.,* 2010. Controle do *Huanglongbing* no estado de São Paulo, Brasil. *Citrus Research & Technology* **31**, 53–64.

Belasque J, Bassanezi RB, Yamamoto PT *et al.*, 2010. Lessons from *Huanglongbing* management in São Paulo State, Brazil. *Journal of Plant Pathology* **92**, 285–302.

Belasque J, Bergamin Filho A, Bassanezi RB *et al.*, 2009. Base científica para a erradicação de plantas sintomáticas e assintomáticas de *Huanglongbing* (HLB, Greening) visando o controle efetivo da doença. *Tropical Plant Pathology* **34**, 137–145.

Bergamin Filho A, Hau B, Amorim L *et al.,* 2002. Análise espacial de epidemias. *Revisão Anual de Patologia de Plantas* **11**.

Bergamin Filho A, Inoue-Nagata AK, Bassanezi RB *et al.*, 2016. The importance of primary inoculum and area-wide disease management to crop health and food security. *Food Security* **8**, 221–238.

Boina DR, Bloomquist JR, 2015. Chemical control of the Asian citrus psyllid and of *Huanglongbing* disease in citrus. *Pest Management Science* **71**, 808–823.

Boina DR, Meyer WL, Onagbola EO *et al.,* 2009. Quantifying dispersal of *Diaphorina citri* (Hemiptera: Psyllidae) by immunomarking and potential impact of unmanaged groves on commercial citrus management. *Environmental entomology* **38**, 1250–1258.

Bové JM, 2006a. *Huanglongbing*: A destructive, newly-emerging, century-old disease of citrus. *Journal of Plant Pathology* **88**, 7–37.

Bové JM, 2014. *Huanglongbing* or yellow shoot, a disease of Gondwanan origin: Will it destroy citrus worldwide? *Phytoparasitica* **42**, 579–583.

Capoor SM, Rao SP, Viswanath DG, 1974. Greening disease of citrus in the Deccan Trap Country and its relationship with the vector, *Diaphorina citri* Kuwayama. In: *Weathers LG, Cohen M. (eds) Proceedings of 6th Conference of International Organization of Citrus Virologists.* 43–49.

CDA (Defesa Agropecuaria do Estado de São Paulo) Portaria CDA - 5, de 3/2/2005: Medidas de Defesa Sanitária Vegetal e Certificação de Conformidade Fitossanitária de Mudas Cítricas no Estado de São Paulo. *Defesa Agropecuaria do Estado de São Paulo*.

CDA (Defesa Agropecuaria do Estado de São Paulo), 2017. Compilação dos dados dos relatorios semestrais do greening enviados para a coordenadoria de Defesa Agropecuaria do Estado de São Paulo (CDA). *Dados da Citricultura Paulista*.

Chong JH, Roda AL, Mannion CM, 2010. Density and natural enemies of the Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Psyllidae), in the residential landscape of Southern Florida. *Journal of Agricultural and Urban Entomology* **27**, 33–49.

Coletta Filho HD, Targon MLPN, Takita MA *et al.,* 2004. First report of the causal agent of *Huanglongbing* ('*Candidatus* Liberibacter asiaticus') in Brazil. *Plant Disease* **88**, 1382.

Costa MG, Barbosa JC, Yamamoto PT *et al.,* 2010. Spatial distribution of *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) in citrus orchards. *Scientia Agricola* **67**, 546–554.

Costa Lima AM, 1942. *Insetos do Brasil, Homoptera.* Escola Nacional de Agronomia. Série Didática **4**.

Cumming GS, George A, 2009. Historical influences dominate the composition of regenerating plant communities in abandoned citrus groves in North-central Florida. *Landscape Ecology* **24**, 957–970.

DAS AK, 2004. Rapid detection of '*Candidatus* Liberibacter asiaticus', the bacterium associated with citrus *Huanglongbing* (Greening) disease using PCR. *Current Science* **87**, 1183–1185.

Davis MJ, Mondal SN, Chen H *et al.,* 2008. Co-cultivation of '*Candidatus* Liberibacter asiaticus' with Actinobacteria from Citrus with *Huanglongbing*. *Plant Disease* **92**, 1547–1550.

Deng X, Lan Y, Hong T *et al.,* 2016. Citrus greening detection using visible spectrum imaging and C-SVC. *Computers and Electronics in Agriculture* **130**, 177–183.

Duran-Vila N, Bové JM, 2015. Citrus HLB is an emerging disease transmitted by psyllid vectors. Can it be prevented? If not, can it be managed? *CIHEAM Watch Letter* **33**.

Etxeberria E, Gonzalez P, Achor D *et al.,* 2009. Anatomical distribution of abnormally high levels of starch in HLB-affected Valencia orange trees. *Physiological and Molecular Plant Pathology* **74**, 76–83.

Farnsworth D, Grogan KA, Van Bruggen AHC *et al.,* 2014. The potential economic cost and response to Greening in Florida citrus.*The magazine of food, farm, and resource issues* **29.**

Folimonova SY, Robertson CJ, Garnsey SM *et al.,* 2009. Examination of the responses of different genotypes of citrus to *Huanglongbing* (Citrus Greening) under different onditions. *Phytopathology* **99**, 1346–1354.

Fundecitrus, 2017. Fundecitrus lança campanha "Unidos contra o greening" para incentivar o controle da pior doença da citricultura. *Fundo de Defesa da Citricultura – FUNDECITRUS*. Available in: https://www.fundecitrus.com.br/comunicacao/noticias

Fundecitrus, 2018a. Levantamento da incidência das doenças dos citros: Greening, CVC e Cancro cítrico no cinturão citrícola de São Paulo e triângulo/sudoeste mineiro. *Fundo de Defesa da Citricultura – FUNDECITRUS*.
Available in: https://www.fundecitrus.com.br/levantamentos/greening

Fundecitrus, 2018b. Phytosanitary Warning System. *Fundo de Defesa da Citricultura – FUNDECITRUS*. Available in: https://www.fundecitrus.com.br/alerta-fitossanitario

Gasparoto MCG, Hau B, Bassanezi RB *et al.,* 2018. Spatiotemporal dynamics of citrus *Huanglongbing* spread: a case study. *Plant Pathology* **67**, 1621–1628.

George J, Ammar E-D, Hall DG *et al.*, 2017. Sclerenchymatous ring as a barrier to phloem feeding by Asian citrus psyllid: Evidence from electrical penetration graph and visualization of stylet pathways. *Plos One* **12**, e0173520.

Godfrey KE, Galindo C, Patt JM *et al.,* 2013. Evaluation of color and scent attractants used to trap and detect Asian citrus psyllid (Hemiptera: Liviidae) in urban environments. *Florida Entomologist* **96**, 1406–1416.

Gómez-Torres ML, Nava DE, Parra JRP *et al.,* 2012. Life table of *Tamarixia radiata* (Hymenoptera: Eulophidae) on *Diaphorina citri* (Hemiptera: Psyllidae) at different temperatures. *Journal of economic entomology* **105**, 338–43.

Gottwald TR, Irey M, Bergamin Filho A *et al.,* 2008. A stochastic spatiotemporal analysis of the contribution of primary versus secondary spread of HLB. In: *Proc. International Research Conference on Huanglongbing*. 247–252.

Gottwald TR, Aubert B, X-YZ, 1989. Preliminary analysis of citrus Greening (*Huanglongbing*) epidemics in the People's Republic of China and French Reunion Island. *Ecology and Epidemiology* **79**, 687–693.

Gottwald TR, Aubert B, 1991. Spatial pattern analysis of citrus Greening In Shantou, China. In: *11 IOCV Conference*. 421–427.

Gottwald TR, 1989. Analysis of disease progress of Citrus Canker in nurseries in Argentina. *Phytopathology* **79**, 1276.

Gottwald TR, Gonzales CI, Mercado BG, 1991. Analysis of the distribution of citrus Greening in groves in the Philippines. In: *11 IOCV Conference: Greening, Stubborn and other diseases*. 414–420.

Gottwald TR, 2007. Citrus canker and citrus *Huanglongbing*, two exotic bacterial diseases threatening the citrus industries of the Western Hemisphere. *Outlooks on Pest Management* **18**, 274–279.

Gottwald TR, 2010b. Current Epidemiological Understanding on Citrus *Huanglongbing*. *Annual review of phytopathology* **48**, 119–139.

Gottwald TR, Graça JV da, Bassanezi RB, 2007. Citrus *Huanglongbing*: The pathogen and its impact. *Plant Health Progress* **36**.

Gottwald TR, Irey M, Gast T, 2008a. The plantation edge effect of HLB. *International Research Conference on Huanglongbing*, 305–308.

Gottwald TR, Irey M, Gast T *et al.*, 2010a. Spatio-temporal analysis of an HLB epidemic in Florida and implications for spread. *Proceedings of the 17 th Conference of International Organization of Citrus Virologists, IOCV*, 84–97.

Gottwald TR, Irey M, Gast T *et al.,* 2008b. HLB Survival Analysis - A spatiotemperal assessment of the threat of an HLB-positive tree to its neighbors. *International Research Conference on Huanglongbing Proceedings*, 291–295.

Gottwald TR, Sun X, Riley T *et al.,* 2002. Geo-referenced spatiotemporal analysis of the urban citrus canker epidemic in Florida. *Phytopathology* **92**, 361–377.

da Graça JV, Douhan GW, Halbert SE *et al.*, 2016. *Huanglongbing*: An overview of a complex pathosystem ravaging the world's citrus. *Journal of Integrative Plant Biology* **58**, 373–387.

Grafton-Cardwell EE, Stelinski LL, Stansly PA, 2013. Biology and management of Asian citrus psyllid, vector of the *Huanglongbing* pathogens. *Annual Review of Entomology* **58**, 413–432.

Halbert SE, Manjunath KL, Roka F *et al.,* 2008. *Huanglongbing* (citrus greening) in Florida, 2008. In: *FFTC-PPRI-NIFTS Joint Workshop on management of citrus greening and virus diseases for the rehabilitation of citrus industry in the ASPAC*. 58–67.

Halbert SE, Manjunath KL, 2004. Asian citrus psyllids (Sternorrhyncha: Psyllidae) and greening disease of citrus: a literature review and assessment of risk in Florida. *Florida Entomologist* **87**, 330–353.

Halbert SE, Manjunath K, Ramadugu C *et al.,* 2012. Incidence of *Huanglongbing*-associated '*Candidatus* Liberibacter Asiaticus' in *Diaphorina citri* (Hemiptera: Psyllidae) collected from plants for sale in Florida. *Florida Entomologist* **95**, 617–624.

Halbert SE, Sun X DW, 2001. Asian citrus psyllid update. Available in: http://www.freshfromflorida.com/pi/enpp/ento/ asian-citrus-psyllid.htm

Halbert SE, 1998. Entomology section. *Tri-ology* **37**.

Hall DG, Albrigo LG, 2007. Estimating the relative abundance of flush shoots in citrus with implications on monitoring insects associated with flush. *HortScience* **42**, 364–368.

Hall DG, Wenninger EJ, Hentz MG, 2011. Temperature studies with the Asian citrus psyllid, *Diaphorina citri*: cold hardiness and temperature thresholds for oviposition. *Journal of Insect Science* **11**, 1–15.

Hendricks KE, Christman M, Roberts PD, 2017. Spatial and temporal patterns of commercial citrus trees affected by phyllosticta citricarpa in Florida. *Scientific Reports* **7**, 1–11.

Hodges AW, Spreen TH, 2012. Economic impacts of Citrus Greening (HLB) in Florida, 2006/07-2010/11. Food and resource economics department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Gainesville.

Huang CHM, Tsai Y, WCL, 1984. Transmission of citrus likubin by a psyllid, *Diaphorina citri*. *Agricultural Research China* **33**, 65–72.

Inoue H, Ohnishi J, Ito T *et al.*, 2009. Enhanced proliferation and efficient transmission of '*Candidatus* Liberibacter asiaticus' by adult *Diaphorina citri* after acquisition feeding in the nymphal stage. *Annals of Applied Biology* **155**, 29–36.

Institute of Food and Agricultural Sciences, 2016. Impact of Citrus Greening on Citrus Operations in Florida **4**.

Kim J-S, Sagaram US, Burns JK *et al*., 2009. Response of Sweet Orange (*Citrus sinensis*) to '*Candidatus* Liberibacter asiaticus' Infection: Microscopy and Microarray Analyses. *Phytopathology* **99**, 50–57.

Kobori Y, Nakata T, Ohto Y *et al*., 2011. Dispersal of adult Asian citrus psyllid, *Diaphorina citri* Kuwayama (Homoptera: Psyllidae), the vector of citrus greening disease, in artificial release experiments. *Applied Entomology and Zoology* **46**, 27–30.

Leal RM, Barbosa JC, Costa MG *et al.*, 2010. Distribuição espacial de *Huanglongbing* (Greening) em citros utilizando a geoestatística. *Revista Brasileira de Fruticultura* **32**, 808–818.

Lee JA, Halbert SE, Dawson WO *et al.*, 2015. Asymptomatic spread of *Huanglongbing* and implications for disease control. *Proceedings of the National Academy of Sciences* **112**, 7605–7610.

Lewis-Rosenblum H, Martini X, Tiwari S *et al.,* 2015. Seasonal movement patterns and long-range dispersal of Asian citrus psyllid in Florida citrus. *Journal of Economic Entomology* **108**, 3–10.

Lin K-H, 1956. Observations on yellow shoot of Citrus*. Acta Phytopathologica Sinica* **2***, 1–42.*

Liu YH, Tsai JH, 2000. Effects of temperature on biology and life table parameters of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Homoptera: Psyllidae). *Annals of Applied Biology* **137**, 201–206.

Lopes SA, Martins EC, Frare GF, 2005. Detecção de '*Candidatus* Liberibacter americanus' em *Murraya paniculata*. *Summa Phytopathologica* **31**, 48–49.

Lopes SA, Martins EC, Frare GF, 2006. Detecção de '*Candidatus* Liberibacter asiaticus' em *Murraya paniculata*. *Fitopatologia Brasileira* **31**, 303.

Lopes SA, Bertolini E, Frare GF *et al.*, 2009a. Graft transmission efficiencies and multiplication of '*Candidatus* Liberibacter americanus' and '*Ca.* Liberibacter asiaticus' in citrus plants. *Phytopathology* **99**, 301–306.

Lopes SA, Frare GF, Bertolini E *et al.*, 2009b. Liberibacters associated with citrus *Huanglongbing* in Brazil: '*Candidatus* Liberibacter asiaticus' is heat tolerant, '*Ca.* L. americanus' is heat sensitive. *Plant Disease* **93**, 257–262.

Martini X, Addison T, Fleming B *et al.,* 2013. Occurrence of *Diaphorina citri* (Hemiptera: Liviidae) in an unexpected ecosystem: The Lake Kissimmee State Park Forest, Florida. *Florida Entomologist* **96**, 658–660.

Martini X, Hoyte A, Stelinski LL, 2014. Abdominal color of the Asian citrus psyllid (Hemiptera: Liviidae) is associated with flight capabilities. *Annals of the Entomological Society of America* **107**, 842–847.

Martini X, Pelz-Stelinski KS, Stelinski LL, 2015. Absence of windbreaks and replanting citrus in solid sets increase density of Asian citrus psyllid populations. *Agriculture, Ecosystems and Environment* **212**, 168–174.

Miranda MP, dos Santos FL, Bassanezi RB *et al.*, 2018. Monitoring methods for *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) on citrus groves with different insecticide application programmes. *Journal of Applied Entomology* **142**, 89–96.

Mishra A, Karimi D, Ehsani R *et al.*, 2011. Evaluation of an active optical sensor for detection of *Huanglongbing* (HLB) disease. *Biosystems Engineering* **110**, 302–309.

Monteiro AB, Pereira De M, 2013. Efeito de um pomar sem manejo de *Huanglongbing* sobre a ocorrência de *Diaphorina citri* e incidência de plantas doentes em uma área vizinha com manejo da doença. Dissertação de mestrado. Fundecitrus.

Nava DE, Torres MLG, Rodrigues MDL*et al.*, 2007. Biology of *Diaphorina citri* (Hem., Psyllidae) on different hosts and at different temperatures. *Journal of Applied Entomology* **131**, 709–715.

Orce IG, Sendín LN, Marano MR *et al.*, 2015. Novel set of real-time PCR primers for simultaneous detection of Liberibacter species associated with citrus *Huanglongbing*. *Scientia Agricola* **72**, 252–259.

Parnell S, Gottwald TR, Gilligan CA *et al.*, 2010. The effect of landscape pattern on the optimal eradication zone of an invading epidemic. *Phytopathology* **100**, 638–644.

Parry M, Gibson GJ, Parnell S *et al.*, 2014. Bayesian inference for an emerging arboreal epidemic in the presence of control. *Proceedings of the National Academy of Sciences of the United States of America* **111**, 6258–62.

Patt JM, Sétamou M, 2010. Responses of the Asian citrus psyllid to volatiles emitted by the flushing shoots of its rutaceous host plants. *Environmental Entomology* **39**, 618–624.

Patt J, Stockton D, Meikle W*et al.*, 2014. Innate and conditioned responses to chemosensory and visual cues in Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Liviidae), vector of *Huanglongbing* pathogens. *Insects* **5**, 921–941.

Pelz-Stelinski KS, Brlansky RH, Ebert TA *et al.*, 2010. Transmission parameters for '*Candidatus* liberibacter asiaticus' by Asian citrus psyllid (Hemiptera: Psyllidae). *Journal of Economic Entomology* **103**, 1531–1541.

Pérez FJM, Flores-Sanchez JL, Monge SD, 2016. Análisis espacial y temporal del *Huanglongbing* de los cítricos (HLB), en el municipio de Xalisco, Nayarit. Tesis, Universidad Autónoma Chapingo.

Ripley BD, 1981. Spatial statistics. *Wiley Series in Probability and Statistics.*

Rogers EM, Shawer DB, 2007. Effectiveness ofseveral soil-applied systemic insecticides for managingthe Asian citrus psyllid, *Diaphorina citri*Kuwayama (Ho-moptera: Psyllidae). *Proc. Fla. State Hortic. Soc* **120**, 116–119.

Sétamou M, da Graça JV, Kunta M, 2014. First Detection of *Huanglongbing* and implementation of its mitigation efforts in Texas. In: *3rd International Research Conference on Huanglongbing.*

Sétamou M, Bartels DW, 2015. Living on the edges: Spatial niche occupation of Asian citrus psyllid, *Diaphorina citri* kuwayama (Hemiptera: Liviidae), in citrus groves. *Plos One* **10**, e0131917.

Sétamou M, Rodriguez D, Saldana R *et al.*, 2010. Efficacy and uptake of soil-applied imidacloprid in the control of Asian citrus psyllid and a citrus leafminer, two foliar-feeding citrus pests. *Journal of economic entomology* **103**, 1711–9.

Sétamou M, Simpson CR, Alabi OJ *et al.*, 2016. Quality Matters: Influences of citrus flush physicochemical characteristics on population dynamics of the Asian citrus psyllid (Hemiptera: Liviidae). *Plos One* **11**, e0168997.

Shen W, Cevallos-Cevallos JM, Nunes da Rocha U *et al.*, 2013a. Relation between plant nutrition, hormones, insecticide applications, bacterial endophytes, and '*Candidatus* Liberibacter' Ct values in citrus trees infected with *Huanglongbing*. *European Journal of Plant Pathology* **137**, 727–742.

Shen W, Halbert SE, Dickstein E *et al.*, 2013b. Occurrence and in-grove distribution of citrus *Huanglongbing* in north central Florida. *Journal of Plant Pathology* **95**, 361–371.

Singerman A, Useche P, 2016. Impact of citrus Greening on citrus operations in surveying the Florida citrus survey-based estimates on HLB. *IFAS Extension*, 2–5.

Skelley LH, Hoy MA, 2004. A synchronous rearing method for the Asian citrus psyllid and its parasitoids in quarantine. *Biological Control* **29**, 14–23.

Spósito MB, Amorim L, Ribeiro PJ *et al.*, 2007. Spatial pattern of trees affected by Black spot in citrus groves in Brazil. *Plant Disease* **91**, 36–40.

Spreen TH, Baldwin J-P, 2013. The impact of *Huanglongbing* (HLB) on citrus tree planting in Florida. *Proceedings of the Southern Agricultural Economics Association Conference*, Orlando, Florida.

Taylor LR, 1984. Assessing and interpreting the spatial distributions of insect populations. *Annual Review of Entomology* **29**, 321–357.

Teixeira DDC, Danet JL, Eveillard S *et al.*, 2005. Citrus *Huanglongbing* in São Paulo State, Brazil: PCR detection of the '*Candidatus*Liberibacter' species associated with the disease. *Molecular and Cellular Probes* **19**, 173–179.

Tiwari S, Lewis-Rosenblum H, Pelz-Stelinski K *et al.*, 2010. Incidence of '*Candidatus* Liberibacter asiaticus' infection in abandoned citrus occurring in proximity to commercially managed groves. *Journal of economic entomology* **103**, 1972–1978.

Tomaseto AF, Krugner R, Lopes JRS, 2015. Effect of plant barriers and citrus leaf age on dispersal of *Diaphorina citri* (Hemiptera: Liviidae). *Journal of Applied Entomology* **140**, 91–102.

Tomaseto AF, Miranda MP, Moral RA *et al.*, 2017. Environmental conditions for *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) take-off. *Journal of Applied Entomology* **142**, 104–113.

Tsai JH, Liu YH, 2000. Biology of *Diaphorina citri* (Homoptera: Psyllidae) on four host plants. *Journal of economic entomology* **93**, 1721–5.

USDA, 2017. Florida Citrus Statistics 2015-2016. *USDA, National Agricultural Statistics Service*.

Valdés RA, Delgado Ortiz JC, Beltrán Beache M *et al.*, 2016. A review of techniques for detecting *Huanglongbing* (greening) in citrus. *Canadian Journal of Microbiology* **62**, 803–811.

Wenninger EJ, Stelinski LL, Hall DG, 2009. Roles of olfactory cues, visual cues, and mating status in orientation of *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) to four different host plants. *Environmental entomology* **38**, 225–34.

Xu CF, Xia YH, Li KB *et al*., 1988. Further study of the transmission of citrus *Huanglongbing* by a psyllid, *Diaphorina citri* Kuwayama. In: *Timmer LW, Garnsey SM, Navarro L. (eds) Proc of the 10th Conf Int Organ Citrus Virol.* 243–248.

Yasuda K, Kawamura F, Oishi T, 2005. Location and preference of adult Asian citrus psyllid, *Diaphorina citri* (Homoptera: Psyllidae) on Chinese Box Orange Jasmine, *Murraya exotica* L. and Flat Lemon, *Citrus depressa*. *Japanese Journal of Applied Entomology and Zoology* **49**, 146–149.

Zhang M, Guo Y, Powell CA *et al.*, 2014. Effective antibiotics against '*Candidatus* Liberibacter asiaticus' in HLB-affected citrus plants identified via the graft-based evaluation. *Plos One* **9**, e111032.

3. SPATIAL PATTERN OF *HUANGLONGBING* EPIDEMIC UNDER STRICT MANAGEMENT IN SÃO PAULO, BRAZIL

Huanglongbing (HLB) is a complex citrus disease with a difficult and expensive management that have been concerning the agribusiness chain of citrus in many countries. Quantifying the spatial nature of the disease spread can be a key to understanding the epidemiology of the pathogen and improve control measurements. This study aimed to determine the spatial pattern of HLB in citrus blocks under strict disease management (trees eradication and vector control), belonging to three properties, in São Paulo State, Brazil. For this, modified Ripley's K-function and kernel density estimation maps (KDE) were performed in 24 commercial citrus blocks (totaling 537.4 ha) where the spatial distribution of infection was assessed 3 to 5 times each, between 2013 and 2017. Cumulative HLB incidence on the 24 blocks ranged from 0.7 to 23.4%. Results of the modified Ripley's K analysis demonstrated a regular distribution pattern of HLB infected trees prevailing in the range of distances studied (up to 434.0 m). Weak aggregation, with an average range of spatial dependency (RSD) between infected trees of 58.0 m, was observed on the 24 citrus blocks. The MAX distance, i.e., the most common distance between pairs of HLB infected trees was 38.1 m for blocks located internally and 51.0 m at the periphery of the properties. Higher averages of RSD and *D. citri* adults per trap were found in blocks located in the periphery of the properties. The KDE found higher density of HLB infected trees at the border of the citrus blocks, mainly at the periphery of the properties. These results demonstrated that, under strict management, HLB primary spread from surrounding areas was predominating on the 24 citrus blocks. Therefore, to reduce new infections, HLB management needs to effective reduce external inoculum sources and/or include strategies that reduce the entry of *D. citri* in the area.

Keywords: Ripley's K-function; Edge effect; Regional management

3.1 Introduction

Huanglongbing (HLB) is considered the most devastating disease of citrus and is present in most of the producing regions worldwide, including the three major citrus-producing countries: Brazil, the USA and China (Bové, 2014). Where it occurs ,HLB has been responsible for drastic reduction of citrus production and quality, citrus blocks abandonment, shortening the life span of citrus orchards and for increasing production costs (Gottwald *et al*., 2010; Farnsworth *et al*., 2014; Das, 2004). In Brazil, it was estimated that more than 40 million trees had been removed by 2016 (CDA, 2017) and 35,3 million orange trees (18.15%) are currently HLB symptomatic in the Citrus Belt area, main orange juice producer region in Brazil (Fundecitrus, 2018a).

There is no commercial variety resistant to HLB or viable curative control methods yet, and the integrated management is the most efficient way to reduce HLB effects. Three basic strategies has been indicated and performed in Brazilian properties, at different levels, since 2004 (Bové, 2006): planting of healthy nursery trees, inspections and eradication of symptomatic trees and frequent chemical control of the vector, the Asian citrus psyllid (ACP), *Diaphorina citri*. Among the strategies to control *D. citri*, insecticides sprays are the most effective and frequently used (Boina & Bloomquist, 2015).

However, even applying the indicated management, HLB can infect 100% of trees in 3 to 5 years (Bassanezi *et al*., 2013; Belasque *et al*., 2010).The indicated management can be very efficient against acquisition and transmission of the pathogen by the vector inside the property (secondary spread) when applied locally. On the other hand, these strategies can have little or no effect on the entrance on ACP that acquired the pathogen at inoculum sources around the property (primary spread) (Gatineau *et al.*, 2010; Bassanezi *et al.*, 2013a,b). The relevance of primary spread on HLB epidemiology is related to the ACP constant movement among citrus groves over short (<200m) and long (>2 km) distances (Boina *et al*., 2009; Lewis-Rosenblum *et al*., 2015; Martini *et al*., 2013, 2014). Thus, even properties performing intensive HLB management are constantly re-infested and subject to continuous primary infections, especially in the borders of the groves (Bergamin Filho *et al.*, 2016).

The border effect is the result of the migration of infective psyllids from outside the grove and can represent from a small portion to a total area of a property, depending on groves shape and size. To reduce the higher incidences of HLB near the periphery areas of the properties, the area-wide management is necessary (Bassanezi *et al*., 2013b; Bergamin Filho *et al*., 2016; Gasparoto *et al*., 2018). Also, many citrus growers have been intensifying the chemical management in the border of the groves by increasing the frequency of insecticide applications in an attempt to reduce HLB incidences in these areas (Miranda *et al.*, 2017). However, the efficiency of these applications is questionable since it effect on the reduction of HLB infected trees on the borders is unknown.

HLB is a complex disease and so, requires complex management measures. Epidemiological analyses are important tools that can bring valuable information on the understanding of complex pathosystems, such as HLB. Quantifying the spatial nature of spread of the associate bacterium can be a key to understanding the epidemiology of the disease, which can then be used to improve its control. Ripley's K-function analysis has been widely used in the study of disease point pattern (Ripley, 1981), including HLB epidemic in commercial citrus blocks in Florida (Gottwald *et al.*, 2010). By calculating the average density of healthy and diseased trees on a sequence of circles with increasing radius surrounding each tree, Ripley's K-function detect the spatial pattern of the disease.

Despite the complexity in the management, the increasing disease incidences and the concerning situation of the agribusiness chain of citrus, relatively few spatial epidemiology studies

have been conducted in HLB pathosystem. In this study, we analyzed the spatial pattern of HLB epidemic in 24 commercial citrus blocks, totaling an area of 537.4 ha, that were under strict disease management, in São Paulo State. The objective of this study was to determine the spatial pattern of HLB in such conditions and gain some information on disease spread, so better management strategies can be applied.

3.2 Material and Methods

3.2.1 Area and data

Locations of trees eradicated due HLB symptoms was assessed in 24 citrus blocks belonging to three commercial citrus properties (E, A and D) located in an HLB endemic region in São Paulo – Brazil (Figure 1). Data consists of more than 330,000 trees distributed among 24 rectangular citrus blocks, totaling 537.4 ha. All citrus blocks were separated by roads and each one was composed of ~60 rows and ~250 trees/row of sweet orange 'Pera Rio' (*Citrus sinesis* (L.) Osbeck). Citrus blocks characteristics are given in Table 1.

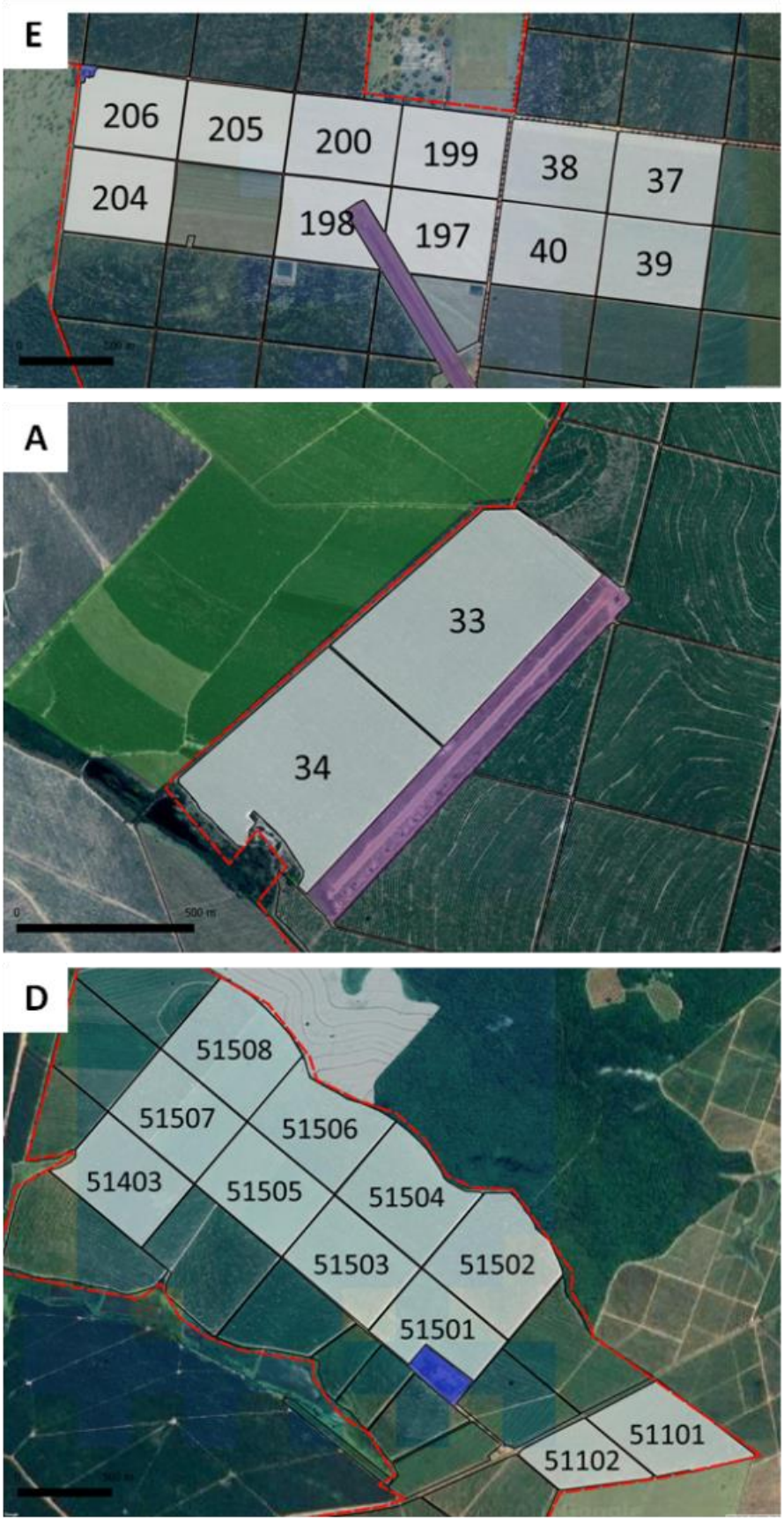

Figure 1. Maps of properties E, A and D and location of studied citrus blocks.Red dashed line represents properties periphery.Lilac, purple and green areas represent facilities, landing strips and citrus neighbors, respectively.

Table 1. Characteristics of 'Pera Rio' sweet orange citrus blocks studied in São Paulo, for HLB eradicated trees spatial pattern.

Property	Block	Size	Rootstock	Spacing between row	Spacing between trees	Date of planting	Total trees
E	37	23.87	Sunki mandarin	7	3	06/2009	11,420
	38	23.61	Sunki mandarin	7	3	06/2009	11,627
	39	25.06	Sunki mandarin	7	3	06/2009	11,417
	40	23.76	Sunki mandarin	7	3	06/2009	11,994
	197	23.07	Citromelo Swingle	6.5	2.2	03/2012	15,792
	198	20.78	Citromelo Swingle	6.5	2.2	03/2012	14,381
	199	24.09	Citromelo Swingle	6.5	2.2	03/2012	16,065
	200	23.81	Citromelo Swingle	6.5	2.2	03/2012	16,047
	204	24.08	Citromelo Swingle	6.5	2.2	06/2012	16,930
	205	24.64	Citromelo Swingle	6.5	2.2	06/2012	16,148
	206	23.2	Citromelo Swingle	6.5	2.2	06/2012	16,210
A	33	24.96	Sunki mandarin	6.5	2	10/2010	19,204
	34	24.41	Sunki mandarin	6.5	2	10/2010	16,563
D	51101	10.52	Regular lime	6.2	2.6	08/2010	5,123
	51102	6.23	Regular lime	6.2	2.6	09/2010	8,610
	51403	25.01	Cleópatra	6.8	2.5	11/2011	13,850
	51501	20.33	Sunki mandarin	6.8	2.3	05/2012	10,537
	51502	32.80	Sunki mandarin	6.8	2.3	10/2012	16,632
	51503	25.00	Sunki mandarin	6.8	2.3	08/2012	16,065
	51504	28.57	Sunki mandarin	6.8	2.3	10/2012	16,380
	51505	24.98	Sunki mandarin	6.8	2.3	11/2012	16,198
	51506	26.05	Sunki mandarin	6.8	2.3	11/2012	14,706
	51507	13.07	Sunki mandarin	6.8	2.3	11/2012	8,460
	51508	15.05	Sunki mandarin	6.8	2.3	12/2012	10,068

Three to five successive evaluations (snapshots) were made, for each block, between 2013 and 2017. Snapshots were based in aerial photographs used to track the disease status of every three of the 24 citrus blocks. Excel binary matrices based on disease status of every tree of each area were prepared with 0 for preset (not symptomatic trees) and 1 for HLB eradicated trees. Each area contains 3 (blocks 37, 38, 39, 40, 51502, 51503, 51504, 51505, 51506, 51507, 51508), 4 (blocks 51101, 51102, 51501, 51403, 197, 198, 199, 200, 204, 205, 206) or 5 (blocks 33 and 34) snapshots, totaling 87 Excel matrices for the 24 blocks. Snapshots were cumulative and therefore were consisted of the new HLB eradicates trees as well as all previous eradicated trees from previous snapshots.

3.2.2 HLB management performed by the properties

The three properties were considered to perform a strict HLB management. For this, inspections and eradication of symptomatic trees and monitoring and chemical control of *D. citri* were performed frequently in properties E, A and D. Replacement of HLB eradicated trees were performed with healthy nursery trees produced in insect-proof nursery. The use of healthy nursery trees in the implementation of the orchards was accomplished by properties E and A. For property D, as reported by property managers, healthy nursery trees were exposed, before being planted and receive chemical management, near to highly infected citrus blocks, for a few days.

At least five inspections per year (average) were performed, by professional scouts, for HLB symptoms detection in canopies of each tree of each block (Table 2). PCR analysis was used for confirmation of infection when the symptomatology was not conclusive. Symptomatic trees were eradicated within 30 days after detection.

Table 2. Inspections per year for HLB symptomatic trees in the 24 citrus blocks.

Farm	Block	Planting	2009	2010	2011	2012	2013	2014	2015	2016*	Average/year
PE	37	2009	2	11	12	10	8	9	7	2	10
	38	2009	3	11	12	9	8	9	7	2	9
	39	2009	4	10	12	10	8	9	5	3	9
	40	2009	4	10	13	9	9	8	6	2	9
	197	2012	-	-	-	5	12	8	5	2	8
	198	2012	-	-	-	4	12	7	3	2	7
	199	2012	-	-	-	3	11	8	5	2	8
	200	2012	-	-	-	4	12	9	4	2	8
	204	2012	-	-	-	2	12	8	4	2	8
	205	2012	-	-	-	2	12	9	4	2	8
	206	2012	-	-	-	2	11	12	4	2	9
PA	33	2009	0	2	11	9	5	8	5	3	7
	34	2009	0	2	11	9	6	8	5	3	7
PD	51101	2010	-	2	12	11	4	6	4	4	7
	51102	2010	-	2	11	11	4	7	4	4	7
	51403	2011	-	-	0	9	6	8	10	5	8
	51501	2012	-	-	-	0	6	6	4	4	5
	51502	2012	-	-	-	1	6	6	3	4	5
	51503	2012	-	-	-	2	6	6	3	4	5
	51504	2012	-	-	-	1	6	5	4	4	5
	51505	2012	-	-	-	1	5	6	3	4	5
	51506	2012	-	-	-	1	6	6	3	4	5
	51507	2012	-	-	-	1	7	6	3	4	5
	51508	2012	-	-	-	0	2	6	3	4	5

* Number of inspections in citrus blocks until Jul 2016

- Year when citrus block wasn't planted yet

D. citri was monitored by observation of eggs, nymphs and adults in branches, by professional scouts, twice a monthly, on 1% of trees, three branches per tree. Also, each block of the properties contained, at least, one yellow adhesive trap (30 x 10 cm) fixed in the upper third of trees located at the border of the block for capture of adult psyllids. Traps were changed and analyzed weekly, by professional scouts. Analyzes of the traps were carried out directly in the field, until Sep 2014, and, after, inside facilities. Data of *D. citri* detection in traps, from Jun 2012 until Oct 2016, was provided by the three properties for this study.

D. citri control was performed with contact insecticides applied via terrestrial, aerial and at the borders of the citrus blocks. Terrestrial insecticide sprays were performed with turbo sprayers, at least twice a month (Table 3), following a pre-established spray program and were intensified according to increasing detection of D. *Citri* or Fundecitrus phytosanitary alert recommendations (Fundecitrus, 2018b). Aerial sprays (5.0 L/ha) were performed with airplanes Ipanema (application range of 18 m) or airplane Air Tractor (application range of 28 m), in the three properties (Table 4). Border insecticide sprays (Table 4) were applied, in all blocks of each property, with thermal foggers oriented from the outside into the blocks. In addition to contact insecticides, systemic insecticides were applied, via soil drench (200 to 1000 ml/tree), in young trees, until 3 years old (Table 5).

Table 3. Terrestrial insecticides sprays performed in the 24 citrus blocks.

Property	Block	Planting	2009	2010	2011	2012	2013	2014	2015	2016*	Average/month
E	37	2009	13	24	32	35	34	28	28	7	2.1
	38	2009	13	24	32	36	34	28	25	8	2.0
	39	2009	12	26	30	36	33	28	19	8	1.9
	40	2009	13	25	32	36	32	27	26	8	2.0
	197	2012	-	-	-	45	47	35	31	10	2.5
	198	2012	-	-	-	48	46	33	31	11	2.5
	199	2012	-	-	-	47	48	35	31	12	2.6
	200	2012	-	-	-	46	46	34	31	11	2.5
	204	2012	-	-	-	27	44	35	34	12	3.2
	205	2012	-	-	-	28	47	35	34	12	3.1
	206	2012	-	-	-	28	47	30	33	12	3.0
A	33	2009	0	6	28	35	35	30	26	12	2.3
	34	2009	0	6	27	35	35	35	31	12	2.4
D	51101	2010	-	0	0	17	35	29	24	11	2.1
	51102	2010	-	0	0	17	36	29	26	9	2.0
	51403	2011	-	-	0	17	37	28	23	10	2.0
	51501	2012	-	-	-	18	36	29	25	12	2.5
	51502	2012	-	-	-	13	35	32	25	11	2.5
	51503	2012	-	-	-	14	37	29	26	9	2.5
	51504	2012	-	-	-	7	39	30	27	10	2.6
	51505	2012	-	-	-	5	37	28	26	9	2.4
	51506	2012	-	-	-	5	35	30	25	9	2.1
	51507	2012	-	-	-	4	36	27	24	9	2.6
	51508	2012	-	-	-	3	34	30	25	13	2.3

* Number of terrestrial sprays until Jul 2016
- Year when citrus block wasn't planted yet

Table 4. Number of aerial and border insecticide sprays in properties PE, PA and PD.

Property	2009	2010	2011	2012	2013	2014	2015	2016*
				Aerial				
E	2	3	9	6	5	7	6	4
A			4	6			5	1
D						3	6	3
				Border				
E							2	1
A			23	32	2	2		
D				23	34	8		

* Number of aerial and border sprays until Jul 2016

Table 5. Number of insecticide application via drench in the 24 citrus blocks.

Property	Block	Planting	2009	2010	2011	2012	2013	2014	2015	2016*
E	37	2009	3	4						
	38	2009	4	3						
	39	2009	3	5						
	40	2009	3	5						
	197	2012	-	-	-	3		1		
	198	2012	-	-	-	4		1		
	199	2012	-	-	-	3		1		
	200	2012	-	-	-	3		1		
	204	2012	-	-	-	2		1		
	205	2012	-	-	-	2		1		
	206	2012	-	-	-	2		1		
A	33	2009	0	0	2	2				
	34	2009	0	0	2	2				
D	51101	2010	-							
	51102	2010	-							
	51403	2011	-	-	0	0	2			
	51501	2012	-	-	-	2	2		1	
	51502	2012	-	-	-	1	2		1	
	51503	2012	-	-	-	1	2		1	
	51504	2012	-	-	-	0	1		1	
	51505	2012	-	-	-	0	2			
	51506	2012	-	-	-	0	1		1	
	51507	2012	-	-	-	0	2			
	51508	2012	-	-	-	0	2			

* Number of applications until Jul 2016
- Year when citrus block wasn't planted yet

3.2.3 Data analysis

Spatial pattern of HLB eradicated trees, of the 87 snapshots belonging to the 24 citrus blocks, was examined via modified Ripley's K-function and kernel density estimation (KDE). For this, the 87 binary matrices based on disease status of the trees were converted in geographical coordinates information. Besides studying HLB spatial pattern in the 24 blocks individually, blocks 37, 38, 39 and 40 were studied grouped as a single area of 98.3 ha, called E2.

Modified Ripley's K-function method was performed as described by Gottwald *et al.* (2002) and Gottwald *et al.* (2010) to indicate the spatial dependence between infected trees through space, i.e., aggregation or regularity. The analysis compared two cumulative distribution functions, one representing the infected trees spatial point patter (cdfi) and the other, the distribution of the total

population of trees (cdft). Both cumulative distribution functions were plotted against the distance of the radius analyzed. The significant difference between cdfi and cdft indicated the range of spatial dependency (RSD), i.e., the range of distance over which there was aggregation of eradicated trees. The analysis considered, for radio distance, the maximum distance to cover the shortest dimension of the block and varied from 77.5 to 434.0 m between the 24 blocks. The RSD and the distance at which maximal spatial difference (Max) occurred were calculated for the 87 snapshots belonging to the 24 citrus blocks, and for E2 area. Kernel density estimation (KDE) maps, applied for each of the 24 citrus blocks, were used to demonstrate where the HLB eradicated trees were concentrated.

Average RSD, HLB cumulative incidences and *D. citri*/trap of the 24 citrus blocks were statically compared by Kruskal-Wallis h test ($p \leq 0.05$) between the properties E, A and D and between the blocks located internally or at the periphery of the properties. Blocks 33, 37, 39, 40, 197, 198, 205, 501, 503, 505, 507 were grouped as internal, and blocks 38, 199, 200, 204, 206, 34, 101, 102, 403, 502, 504, 506, 508 were grouped as peripheral. Internal blocks were located, at least, 200 m away of the periphery of the properties, while peripheral blocks were facing external areas of the properties. Average *D. citri*/trap of all citrus blocks of each property (data of the entire properties) were also compared between properties E, A and D, which were composed of 314, 34 and 35 citrus blocks, respectively.

3.3 Results

Modified Ripley's K-function analysis allowed accessing the range of spatial dependency (RSD) and the distance of maximum departure from randomness (MAX) for successive snapshots of HLB infection spatial distribution in a total area of 537.4 ha, that was under strict disease management. By applying this method to the 3 to 5 successive snapshots of the 24 citrus blocks and E2 area, it was observed a spatial pattern structure of HLB infected weakly related in very short distances and regular distribution pattern prevailed in the range of distances studied (77.5 to 434.0 m) (Figure 2). For the 87 snapshots analyzed, it was observed an average RSD of 58.0 m, ranging from 16.2 (block 200) to 221.0 m (block 204). The average MAX, i.e., the most common distance between pairs of HLB infected trees, was of 45.1 m, ranging from 11.6 (block 198) to 215.1 m (block 204).

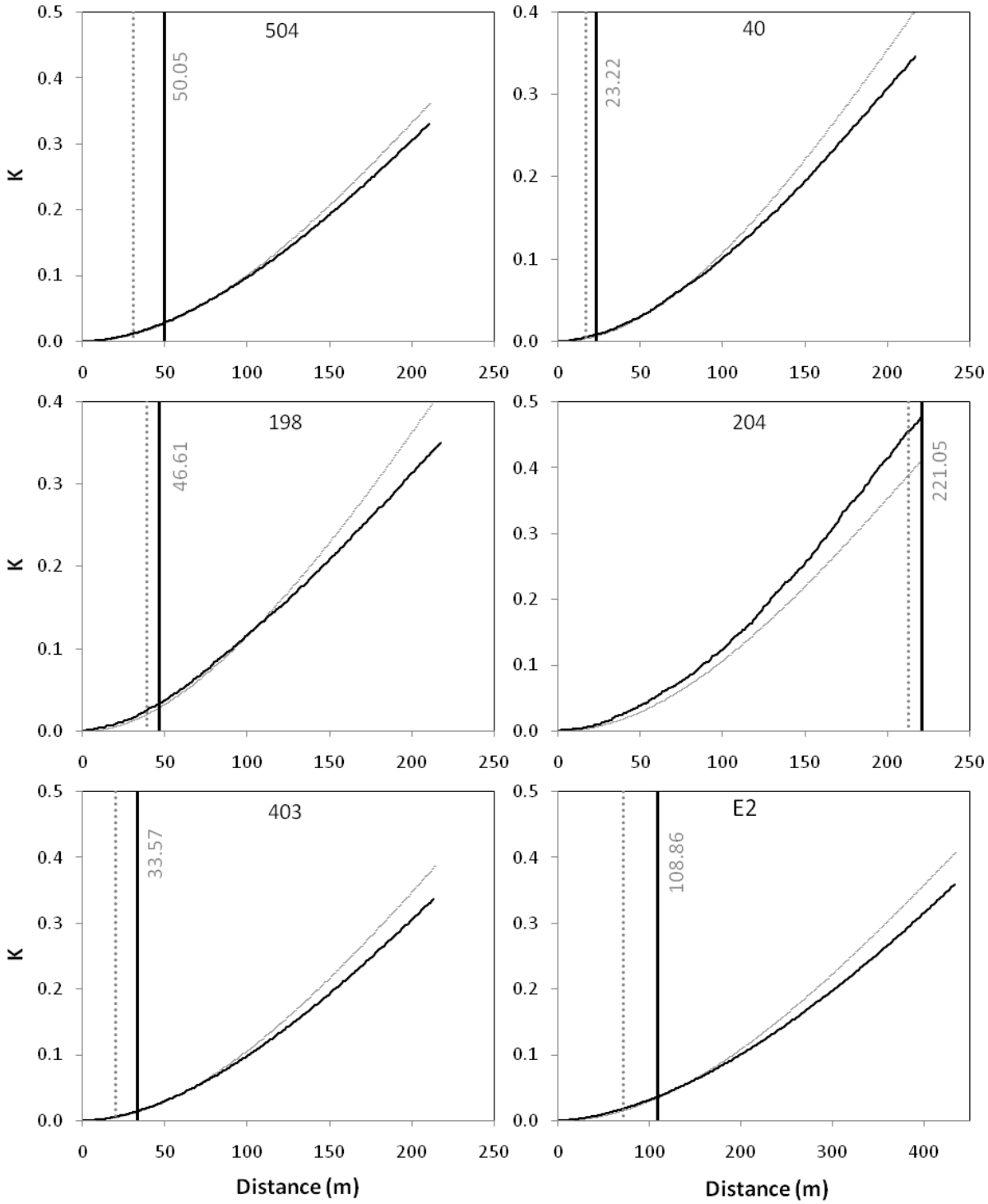

Figure 2. Modified Ripley's K-function analysis of HLB infection spatial pattern in commercial citrus blocks under strict disease management, in São Paulo State. Results of the last snapshot (cumulative incidence) of the blocks 51504, 40, 198, 204, 51403, and area E2 are presented. Infected trees spatial point patter (cdfi) and complete spatial randomness (cdft) are represented by the black and grey curves, respectively. Range of spatial dependency (RSD) and the distance of maximum departure from randomness (MAX) are represented by the solid black and dashed grey lines, respectively.

RSD distances between HLB infected trees, in the cumulative incidences, for all the 24 citrus blocks, are presented in Figure 3. The higher spatial dependency distances were observed in citrus blocks 38 (102.1 m), 204 (221 m), 205 (168.9 m) and 206 (87.6 m), all belonging to property E. Even so, the distances found can be considered very low. Properties E, A and D didn't differ (p>0.05) in average RSD, which were 75.4, 47.5 and 40.1 m, respectively. On the other hand, the 24 blocks differed (p≤0.05) when RSD was compared between internal (46.1 m) and peripheral (68.1 m) blocks. The MAX was 38.1 m and 51.0 m for blocks located internally and at the periphery of the properties, respectively.

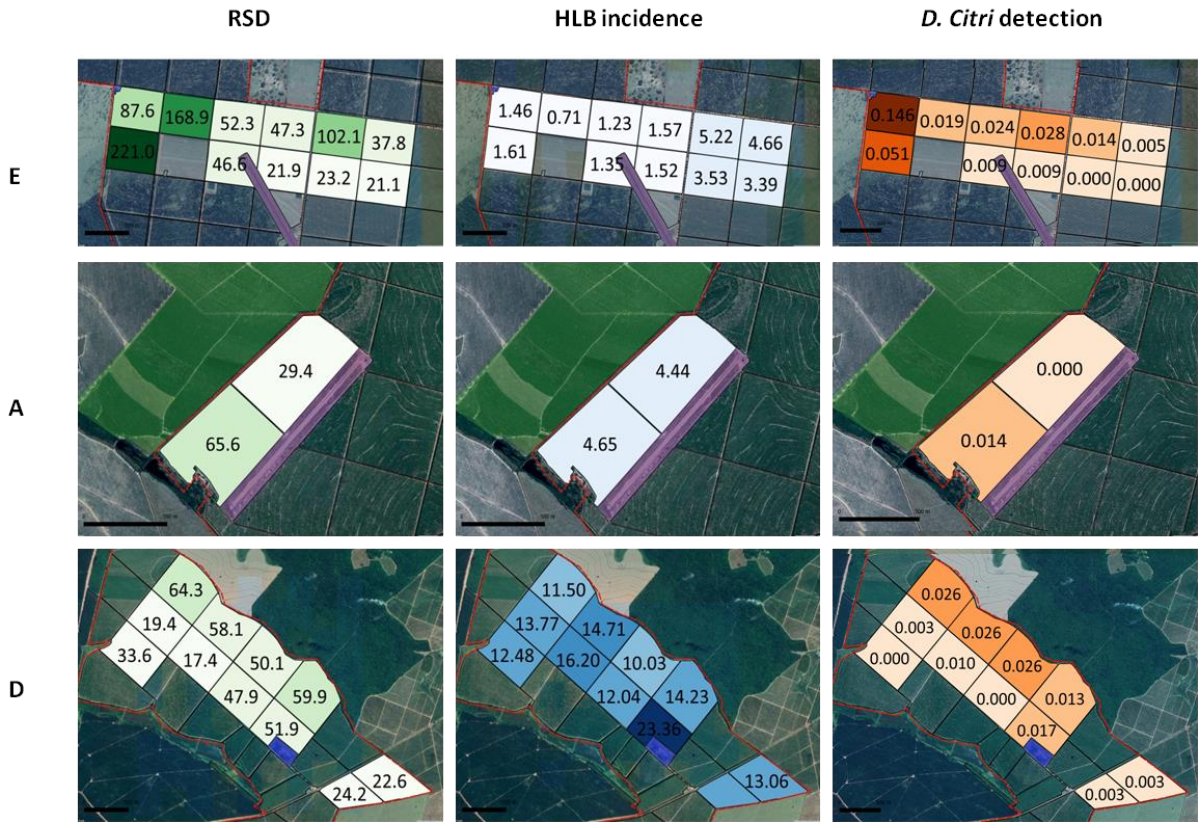

Figure 3. Maps of properties E, A and D with studied citrus blocks. Range of spatial dependency (RSD), HLB incidences and average *D. citri*/trap are presented in green, blue and orange, respectively. Data of *D. citri* detection from Jul 2012 until Oct 2016. Redline represents the property's periphery. Lilac, purple and green areas represent facilities, landing strips and citrus neighbors, respectively.

HLB cumulative incidences ranged from 0.7 to 23.4% of eradicated trees on the 24 citrus blocks (Figure 3). The higher average of HLB incidence was observed in the property D (14.0%) (p≤0.05) when compared with properties E and A (2.4 and 4.5%, respectively). However, the average HLB incidences didn't differ (p>0.05) between the blocks located internally (7.2%) or at the periphery of the properties (8.0%).

The average *D. citri*/trap was studied in two different scales: in the 24 studied citrus blocks and at the entire properties E, A and D. The average *D. citri*/trap in the 24 blocks varied between 0.000 and 0.146 (Figure 3). The blocks 33, 39, 40, 51403 and 51503 didn't have *D. citri* detection in the period studied. No significant differences (p>0.05) were observed between average of *D. citri*/trap of the 24 blocks separated by properties E, A and D, which presented 0.03, 0.01 and 0.01 adults per trap, respectively. When separated by internals or peripherals, the 24 blocks differed (p≤0.05), presenting 0.01 and 0.03 *D. citri*/trap, respectively. The average *D. citri* detection per trap of the entire properties E, A and D was 0.016 (min 0.000 – max 0.146), 0.016 (min 0.000 and max 0.097) and 0.010 (min 0.00 and max 0.043), respectively. Properties didn't differ (p≥0.05) on average *D. citri*/trap.

KDE maps demonstrated higher concentration of HLB eradicated trees in the peripheral areas of the properties (Figure 4). The higher concentrations of eradicated trees seem to be related with external areas, but it was also possible to observe a higher density of eradicated trees near internal roads or areas without citrus trees, indicating the importance of these areas to vector attraction.

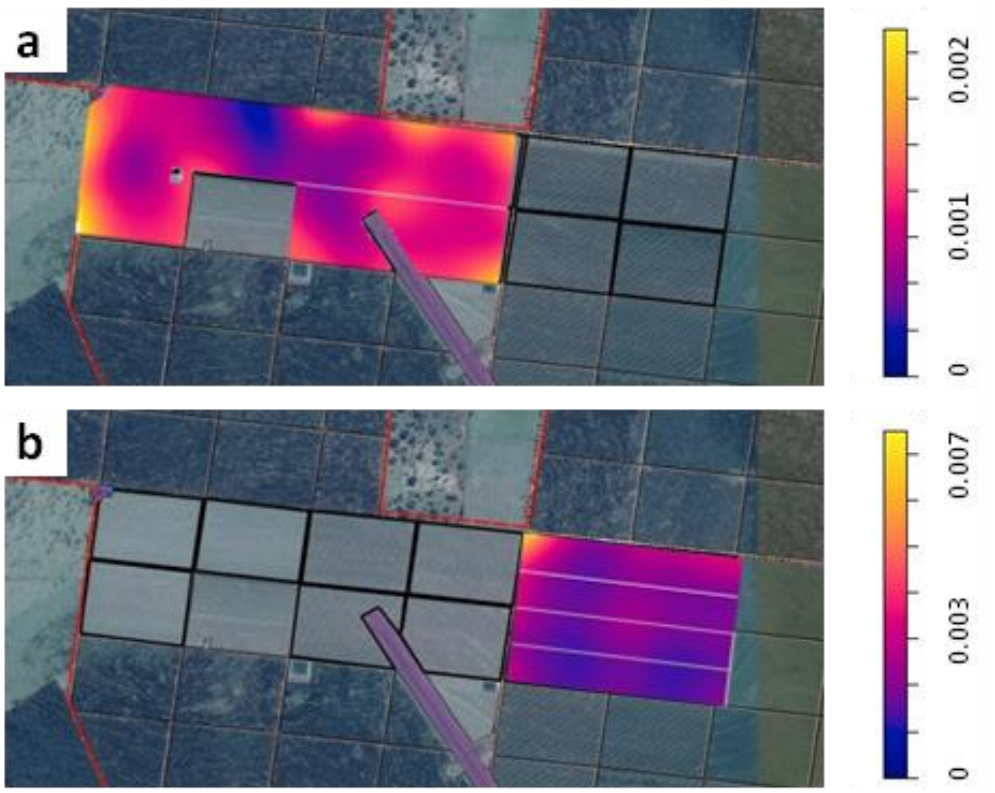

Figure 4. KDE maps of citrus blocks from property E. Citrus blocks planted in 2012 with maximum density of 0.002 (a) and planted in 2009 with maximum density of 0.007 (b). Different colors correspond to ranges of HLB incidence according to the bars on the right side. Red dashed line and lilac area represent property periphery and landing strip, respectively.

3.4 Discussion

The results here presented give strong indications that primary spread from surrounding areas was predominant on the 24 studied citrus blocks (total area of 537.4 ha) under strict HLB management. Results of the modified Ripley's K method and KDE maps demonstrated very short distances of weak spatial dependency between infected trees (average of 58.0 m) occurring in the border area of the citrus block. For most of the distances analyzed, Ripley's K-function indicated, predominantly, a regular distribution of HLB eradicated trees, except for block 204, which presented aggregation distribution for all the distance analyzed (221.05). These results were supported by the higher averages of *D. citri*/trap and RSD distances found in blocks next to external areas rather than in blocks surrounded by citrus, despite the very low averages found. The spatial pattern observed and the strict HLB management performed in the areas indicates that the acquisition and transmission of the bacterium in trees within the orchard, i.e., the secondary spread should be rarely occurring. Results also demonstrated that the infective vectors flying from external inoculum sources (primary spread) land preferably in the first dozen of meters of the citrus blocks. After landing, the infective vector may infect one or a few trees before being killed by the frequent insecticide sprays. The predominance of the primary spread in the studied areas explains why aggregation was not found inside the citrus blocks.

Ripley's K-function was used by Gottwald *et al.* (2010) in the study of HLB spatial point pattern in the Southern Gardens citrus groves (>4,800 ha), in South Florida. The frequency of insecticide sprays applied in Florida, at the time, was 6 times per year (Irey *et al.*, 2008), while in the properties studied, at least 24 sprays per year. Very different from the results here presented, those authors verified a HLB pattern highly spatially related over large distance, with RSD between infected trees in an average distance of 3.5 km. Also, via a stochastic model, they observed that HLB spread occurred as an incessant mixture of primary and secondary infections, i.e., as a continuous introduction of inoculum from outside the area and simultaneous local spread within the area. According to the authors, the level of psyllid population was unprecedented high compared to other recorded psyllid infestations. Thus, the low frequency of psyllid management added to the high vector infestation may have favored the occurrence of secondary infections and, consequently, the strong RSD at very long distances found in Florida when compared to the results found in São Paulo.

In the same study, Gottwald *et al.* (2010) verified the most common distance between pairs of HLB-infected trees (MAX), with a median distance of 1.58 km. These results suggested that there was a spatial relationship that was repeated most frequently at about 1.58 km, and may well indicate a common distance for psyllid dispersal of HLB. Here, we observed MAX distance of 38.1 and 51.0 m

for blocks located internally and at the periphery of the properties, respectively. As these distances were related to the periphery area of the blocks by the KDE maps, they may indicate the most common distance for psyllid landing in a tree after entering the area, i.e., the range in which the most insects occurred.

HLB and *D. citri* higher incidences in peripheral areas receive the name of edge effect, and has been observed by many authors (Bassanezi *et al*., 2013; Bergamin Filho *et al*., 2016; Boina *et al*., 2009; Monteiro *et al*., 2013; Sétamou *et al*., 2015; Sétamou & Bartels, 2015; Tomaseto *et al*., 2015). *D. citri* behavior of frequent dispersion among groves also has being related to the spatial process behind the edge effects (Boina *et al.*, 2009; Gottwald, 2010; Hall & Hentz, 2011). According to Sétamou & Bartels (2015), *D. citri* migration to, preferably, peripheral trees can be related to inter-generational population increase in new flush cycles, the result of migration processes, or differential vector performance on border areas. Regardless of the reasons of *D. citri* preferences for trees near the edge of groves, the edge effect is a strong indication of infective psyllid migration from surrounding areas, and, consequently, of HLB primary infections. Primary infections are responsible for introduction of HLB into new areas and are extremely difficult to prevent, and this is the biggest challenge in managing HLB in areas with strict management, i.e., HLB-tree eradication and vector control (Gottwald *et al.*, 2010).

Besides demonstrating the concentration of infected trees mainly in the periphery area of the properties, with KDE we also demonstrated this patter in the border area of internal citrus blocks that were 200 m away from external areas. This result indicates that the “cloud” of vectors, after departing from an external inoculum source, will preferably land in the first dozen of meters of the first citrus blocks of the property. However, a portion of the population will continue to move into the property and, in turn, will land preferentially near the roads, lamps or facilities. The tendency of HLB infections to concentrate near areas where citrus blocks face internal voids related to roads, canals, containers, houses, buildings and equipment was related by some authors (Bassanezi *et al.*, 2005; Gottwald, 2008; Gasparoto *et al.*, 2018). The higher density of eradicated trees occurring near the contrast between citrus and non citrus areas indicates the importance of these areas to HLB new infections. Also, in the 24 blocks studies, the roads could be acting as a direct link between external areas and the interior of the properties.

Under the conditions in which this study was carried, a weak aggregation of eradicated trees was found at average distances of 58.0 m in the border of the citrus blocks, and a regular distribution prevailed in the areas. These results are indicating that primary spread prevails and the population of *D. citri* will land preferably in the first dozen of meters of the property, but a portion of the vector population will continue to dilute into the property and tend to aggregate near areas of contrast

between citrus and non citrus. Thus, knowing this disease behavior and also the strict management applied in the area, some assumptions can be made related to the disease control strategies.

As a result of the edge effect, many citrus growers have been intuitively increasing the frequency of insecticide sprays on the edges of their properties in an attempt to reduce the number of HLB-diseased plants. This strategy was used in properties A and D, mainly, and for a short period as it wasn't effective (Table 4). This border sprays are usually performed by skirting the periphery of the grove with the spraying equipment facing the trees in the edge, without entering the grove. Producers believe that these border sprays would reduce *D. citri* population and, consequently, disease incidences. However, Asato (2018) verified that border spray presents an effective coverage (≥ 30% of the canopy) only in the first trees on the border of the grove and additional border sprays in intervals of 7 and 14 days weren't efficient to reduce *D. citri* population and HLB symptoms. Based in the results here presents, we believe that even if applied in longer distances, the intensification of insecticide sprays in the border may not be effective to reduce HLB incidences. At first, some of the psyllids that land in the border of the citrus blocks are still able to cause infection before being killed by the insecticide. Then, a proportion of the *D. citri* "cloud" dilute into the property and promote new infections in internal blocks.

According to the founds evidencing the prevalence of primary spread, the main strategy that would effectively reduce HLB incidences in a property already performing a strict internal management, is an external reduction of inoculum sources. Strategies to reduce vector entering on the property, such as the non-eradication of peripheral trees, attractive killer plants or planting parallel to the roads could also help on HLB incidences reduction, but further investigations are needed. The importance of the *D. citri* "cloud" reduction is evidenced by the incidences found in the young citrus blocks here studied (6 to 9 years) under a strict HLB management. The 24 citrus blocks from properties E, A and D had, until 2017, averages of 2.4%, 4.5% and 14.0% of the trees eradicated, respectively. Eradication of HLB symptomatic trees doesn't only mean loss of production, but also expenses with inspections by professional scouts, eradication operations, removal of the eradicated trees and replanting of healthy nursery trees. Furthermore, knowing of the higher incidence of HLB in the periphery of the properties and that contrast areas are more subjected to the occurrence of the disease, planning the planting of new orchard avoiding narrow shaped groves and contrasting areas between the blocks can be considered on orchards renovation.

Average *D. citri*/trap observed in properties E, A and D (0.016, 0.016 and 0.010, respectively), demonstrated a lower vector population compared with studies conducted in other countries and also in Brazil. Hall *et al.* (2008) reported a mean number of 6 and 10 *D. citri*/trap per week in the young and mature trees, respectively, in Florida. In Brazil, Bassanezi *et al.* (2013b) observed 0.08 to

0.46 adults per trap. The frequent insecticide sprays for psyllid control made in São Paulo-Brazil may partially explain the lower *D. citri* population densities in commercial citrus groves.

Different of higher RSD distances and average *D. citri*/trap, peripheral blocks didn't present HLB higher incidences than internal blocks. The low difference between these groups can be related to the intense disease management performed in all the citrus blocks. This also can be related to the very high HLB incidences observed in the blocks of property D, when compared with properties E and A. Property D presented higher incidences in internal than peripheral blocks. Both, property D higher incidence when compared to the other properties and the higher incidences observed in internal blocks, may be related to the exposure of the healthy nursery trees to highly infected citrus blocks, before being planted and receive chemical management, and to the large interval of planting between the citrus blocks (Table 1).

The results here presented highlight the importance of the primary spread to the HLB epidemic and, consequently, the need of external management. Bassanezi *et al*. (2013b) have already demonstrated that an area-wide inoculum reduction and vector control is need for the reduction of the primary spread. Not only properties with poor or no HLB management as also the infected hosts in sidewalks and backyards have been a major problem for efficient HLB management (Bassanezi *et al.*, 2013a,b; Bové, 2014; Michigami *et al*., 2015) and so, these plants should be included on the regional management. Perform an effective reduction of inoculum sources, including non-commercial citrus plants, is a challenge for HLB management in all America continent.

Despite the complexity for HLB management, the increasing disease incidences and the concerning situation of the agribusiness chain of citrus, relatively few spatial epidemiology studies have been conducted about the HLB pathosystem. This study shows, for the first time, the use of Ripley's K- function method for spatial analysis of HLB epidemics in area under frequent HLB-tree eradication and vector control. By the very low *D. citri* populations identified in the yellow stick traps, we demonstrate the effectiveness of the vector control. However, even under these conditions, the properties suffered increase in HLB incidences and had a high amount of trees eradicated due HLB infection. To reduce HLB incidences, citrus groves must keep the strict management in all property area, but implement strategies that reduce *D. citri* entering the property. Thus, *D. citri* frequent monitoring is essential in the indication of external inoculum sources. In addition, efforts must be adopted to reduce the number of citrus and *Murraya* spp. plants maintained without vector control around commercial citrus areas.

REFERENCES

Asato PA, 2018. Eficiência da pulverização de inseticida na borda dos talhões no manejo do H*uanglonglbing*. Dessertação, Fundecitrus.

Bassanezi RB, Belasque J, Montesino LH, 2013a. Frequency of symptomatic trees removal in small citrus blocks on citrus *Huanglongbing* epidemics. *Crop Protection* **52**, 72–77.

Bassanezi RB, Busato LA, Bergamin Filho A *et al.*, 2005. Preliminary spatial pattern analysis of *Huanglongbing* in São Paulo, Brazil. *Sixteenth IOCV Conference* **16**.

Bassanezi RB, Montesino LH, Gimenes-fernandes N *et al.*, 2013b. Efficacy of area-wide inoculum reduction and vector control on temporal progress of *Huanglongbing* in young sweet orange plantings. *Plant Disease* **97**, 789–796.

Belasque J, Bassanezi RB, Yamamoto PT *et al.*, 2010. Lessons from *Huanglongbing* management in São Paulo State, Brazil. *Journal of Plant Pathology* **92**, 285–302.

Bergamin Filho A, Inoue-Nagata AK, Bassanezi RB *et al.*, 2016. The importance of primary inoculum and area-wide disease management to crop health and food security. *Food Security* **8**, 221–238.

Boina DR, Bloomquist JR, 2015. Chemical control of the Asian citrus psyllid and of *Huanglongbing* disease in citrus. *Pest Management Science* **71**, 808–823.

Boina DR, Meyer WL, Onagbola EO *et al.*, 2009. Quantifying dispersal of *Diaphorina citri* (Hemiptera: Psyllidae) by immunomarking and potential impact of unmanaged groves on commercial citrus management. *Environmental entomology* **38**, 1250–1258.

Bové JM, 2006. *Huanglongbing*: A destructive, newly-emerging, century-old disease of citrus. *Journal of Plant Pathology* **88**, 7–37.

Bové JM, 2014. *Huanglongbing* or yellow shoot, a disease of Gondwanan origin: Will it destroy citrus worldwide? *Phytoparasitica* **42**, 579–583.

CDA (Defesa Agropecuaria do Estado de São Paulo), 2017. Compilação dos dados dos relatorios semestrais do greening enviados para a coordenadoria de Defesa Agropecuaria do Estado de São Paulo (CDA). *Dados da Citricultura Paulista*.

DAS AK, 2004. Rapid detection of '*Candidatus* Liberibacter asiaticus', the bacterium associated with citrus *Huanglongbing* (Greening) disease using PCR. *Current Science* **87**, 1183–1185.

Fundecitrus, 2018a. Levantamento da incidência das doenças dos citros: Greening, CVC e Cancro cítrico no cinturão citrícola de São Paulo e triângulo/sudoeste mineiro. *Fundo de Defesa da Citricultura – FUNDECITRUS*.
Available in: https://www.fundecitrus.com.br/levantamentos/greening

Fundecitrus, 2018b. Phytosanitary Warning System. *Fundo de Defesa da Citricultura – FUNDECITRUS*. Available in: https://www.fundecitrus.com.br/alerta-fitossanitario

Farnsworth D, Grogan KA, Van Bruggen AHC *et al.,* 2014. The potential economic cost and response to Greening in Florida citrus.*The magazine of food, farm, and resource issues* **29.**

Gasparoto MCG, Hau B, Bassanezi RB *et al.*, 2018. Spatiotemporal dynamics of citrus *Huanglongbing* spread: a case study. *Plant Pathology* **67**, 1621–1628.

Gatineau F, Bonnot F, Hong Yen TT *et al.*, 2010. Effects of imidacloprid and fenobucarb on the dynamics of the psyllid *Diaphorina citri* Kuwayama and on the incidence of '*Candidatus* Liberibacter asiaticus'. *Fruits* **65**, 209–220.

Gottwald TR, Irey M, Gast T, 2008a. The plantation edge effect of HLB. *International Research Conference on Huanglongbing*, 305–308.

Gottwald TR, 2010. Current epidemiological understanding of citrus *Huanglongbing*. *Annu. Rev. Plant Phytopathol* **48**, 119–139.

Gottwald TR, Irey M, Gast T *et al.*, 2010. Spatio-temporal analysis of an HLB epidemic in Florida and implications for spread. *Proceedings of the 17 th Conference of International Organization of Citrus Virologists, IOCV*, 84–97.

Gottwald TR, Sun X, Riley T *et al.*, 2002. Geo-referenced spatiotemporal analysis of the urban citrus canker epidemic in Florida. *Phytopathology* **92**, 361–377.

Halbert SE, Manjunath KL, 2004. Asian citrus psyllids (Sternorrhyncha: Psyllidae) and Greening disease of citrus: a literature review and assessment of risk in Florida.

Hall DG, Hentz MG, 2011. Seasonal flight activity by the Asian citrus psyllid in east central Florida. *Entomologia Experimentalis et Applicata* **139**, 75–85.

Hall DG, Hentz MG, Adair R, 2008. Population ecology and phenology of *Diaphorina citri* (Hemiptera: Psyllidae) in two Florida citrus groves. *Environmental Entomology* **37**, 914–924.

Irey MS, Gast T, Snively J, 2008.Grove management at Southern Gardens citrus in the presence of Huanglongbing. *I Taller Internacional sobre Huanglongbing de los cítricos ('Candidatus Liberibacter spp') y elpsílido asiático de los cítricos (Diaphorina citri)*.

Lewis-Rosenblum H, Martini X, Tiwari S *et al.*, 2015. Seasonal movement patterns and long-range dispersal of Asian citrus psyllid in Florida citrus. *Journal of Economic Entomology* **108**, 3–10.

Martini X, Addison T, Fleming B *et al.*, 2013. Occurrence of *Diaphorina citri* (Hemiptera: Liviidae) in an Unexpected Ecosystem: The Lake Kissimmee State Park Forest, Florida. *Florida Entomologist* **96**, 658–660.

Martini X, Hoyte A, Stelinski LL, 2014. Abdominal color of the Asian citrus psyllid (Hemiptera: Liviidae) is associated with flight capabilities. *Annals of the Entomological Society of America* **107**, 842–847.

Michigami FAB, Girotto LF, Bassanezi RB, 2015. Effect of internal and external inoculum control practices on HLB epidemic progress in a commercial citrus grove. *Journal of Citrus Pathology* **2**.

Miranda MP, dos Santos FL, Bassanezi RB *et al.*, 2017. Monitoring methods for *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) on citrus groves with different insecticide application programmes. *Journal of Applied Entomology* **142**, 89–96.

Monteiro AB, Pereira De M, Bassanezi RB, 2013. Efeito de um pomar sem manejo de *Huanglongbing* sobre a ocorrência de *Diaphorina citri* e incidência de plantas doentes em uma área vizinha com manejo da doença. Dissertação, Fundecitrus.

Ripley BD, 1981. *Spatial statistics*. New York.

Sétamou M, Bartels DW, 2015. Living on the edges: Spatial niche occupation of asian citrus psyllid, *Diaphorina citri* kuwayama (Hemiptera: Liviidae), in citrus groves. *Plos One* **10**, e0131917.

Tomaseto AF, Krugner R, Lopes JRS, 2015. Effect of plant barriers and citrus leaf age on dispersal of *Diaphorina citri* (Hemiptera: Liviidae). *Journal of Applied Entomology* **140**, 91–102.

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4. TEMPORAL PROGRESS OF *HUANGLONGBING* (HLB) AND SPATIAL DISTRIBUTION OF HLB AFFECTED AREAS AND THE VECTOR *DIAPHORINA CITRI* (HEMIPTERA: LIVIIDAE)

Huanglongbing (HLB) is the main citrus disease and despite many efforts to its control disease incidence continues to increase in São Paulo State, Brazil. The objectives of this study were to better understand HLB epidemics under strict disease management and to demonstrate the importance of external sources of inoculum (primary spread) in HLB epidemics. Our study was carried out in a large commercial citrus property (~8.000 ha, 314 citrus blocks) located in the central area of São Paulo. This property performed strict HLB management (frequent symptomatic trees eradication and vector control). Gompertz and logistic temporal models were fitted to annual disease progress of 177 citrus blocks and average *Diaphorina citri* (ACP) per trap were determined in 296 citrus blocks. Disease progress rate, HLB final incidence and average ACP per trap were compared between blocks located at the periphery (edge) or internally (surrounded by citrus) in the property. The findings, then, were related to external HLB inoculum sources. Both, Gompertz and logistic temporal models fitted well to HLB annual incidences, but Gompertz was chosen as the best model to describe HLB epidemics (higher R^2 and better distribution of residues). Values of Gompertz progress rates (ranging from 0.04 to 0.28 per year), average HLB incidence (0.11), and average ACP per trap (0.016) were low compared with data in the literature. The variables differed ($p \leq 0.05$) between peripheral and internal blocks, higher values being related mainly with blocks in property's periphery. A great amount of noncommercial trees potentially serving as HLB primary inoculum was found near citrus blocks presenting higher rates of disease progress, HLB incidences, and ACP per trap. The regional management of HLB should include, besides commercial orchards, noncommercial trees.

Keywords: Gompertz model; Logistic model; Regional management; External inoculum sources; Noncommercial plants

4.1 Introduction

Huanglongbing (HLB) is a citrus vectored disease associated with three phloem-restricted bacteria '*Candidatus* Liberibacters pp'. In America and Asia continents, epidemics of HLB are associated with '*Ca.* Liberibacter asiaticus' (Clas). In São Paulo State (SPS) - Brazil, also occurs '*Ca.* Liberibacter americanus' (Clam), but with an extremely low frequency compared to Clas. Those bacteria are transmitted by the Asian citrus psyllid (ACP), *Diaphorina citri* (Kuwayama) (Hemiptera: Liviidae). The symptoms associated to HLB include yellow branches sparsely foliated, tree decline, asymmetric and ill-flavored fruits, aborted seeds and fruit drop. There aren't, until now, resistant commercial varieties or curative methods for disease control and the prevention of new infections is

the most efficient way to reduce HLB effects. This preventive management started to be adopted in SPS as soon as HLB was identified, in March 2004 (Coletta Filho *et al*., 2004; Teixeira *et al.*, 2005), and consist of three practices: the use of healthy plants, produced in insect-proof screen-houses; eradication of symptomatic trees; and chemical control of the vector, *D. citri* (Bové, 2006).

HLB account with a long incubation period (from months until years) and disease symptoms can be similar to those associated to nutritional deficiency or other diseases. Thus, for more efficient detection of symptomatic trees, inspections are indicated to be performed by trained and experienced team and with the use of platforms (Belasque *et al*., 2009). Even so, according to Bové (2014), only 60% to 70% of symptomatic trees are detected and those affected, but still asymptomatic, remain as sources of inoculum in the area under strict management. In addition, symptomatic trees detection and eradication are highly cost and, nowadays, it isn't adopted by several growers in SPS.

On the other hand, the chemical control of the vector is a less costly and more accepted practice by the growers. Generally, in SPS, one to four insecticide applications per month are performed in commercial orchards. The vector population is monitored by yellow stick cards (traps) and/or visual inspection of leaves to help growers on the decision about the time and frequency of insecticide sprays (Bassanezi *et al.*, 2013a,b), however the adoption of a fixed calendar schedule is the current practice. There is also a new tool available to help citrus growers on the *D. citri* population control called "Phytosanitary Warning System", developed by a citrus growers association (Fundecitrus, 2018b). This system is based on the counts of thousands of yellow stick cards present in commercial and noncommercial areas in SPS. The population dynamics of adults, and its spatial distribution, are available in a website and warning messages are sent to citrus growers when the vector population increases in a given area. The practical effect of this system are insecticide sprays been applied at the same moment by neighboring citrus growers.

Since the first detection of HLB in SPS, in 2004, citrus growers have eradicated more than 40 million HLB symptomatic trees (CDA, 2017). This amount corresponds to more than 20% of the sweet orange (*Citrus sinensis* L.) trees population in the Citrus Belt area, main orange juice producer region in Brazil (Fundecitrus, 2018a). In addition, in 2018 it was estimated that 35.3 million orange trees (18.15%) were currently HLB symptomatic in this region (Fundecitrus, 2018a). Even carrying a strict management with frequent HLB-symptomatic trees eradication and vector control, new frequent HLB infections occur in any affected farm present in SPS in Brazil. By consequence, more than a thousand of citrus properties are no longer cultivated with sweet oranges since 2015, especially small- and medium-size properties (those with less than fifty thousand trees) (Fundecitrus, 2018a).

D. citri is able to disperse several kilometers without wind assistance (Martini *et al.*, 2013, 2014; Lewis-Rosenblum *et al.*, 2015) and can move frequently from abandoned to managed orchards (Boina *et al.*, 2009; Tiwari *et al.*, 2010; Lewis-Rosenblum *et al.*, 2015). As presented by Bergamin Filho *et al.* (2016), in citrus farms maintained with strict management, new HLB infections occur mainly as a result of the dispersion of infective adults from external sources of inoculum, i.e., primary spread. The frequent HLB-symptomatic trees eradication and the low populations of *D. citri* inside of the managed properties, as result of frequent insecticide sprays, prevent the occurrence of new infections from HLB-affected trees present in the area under control, i.e., secondary spread. However, nymphs and adults can acquire the bacterium from infected plants present in commercial properties with poor management, abandoned areas, backyards or sidewalks, and migrate to orchards under strict management, and then transmit the bacteria to healthy trees before being reached by chemical control.

As a consequence of the primary spread from neighboring properties that don't perform HLB management, most of HLB-eradicated trees are on orchard's edges (edge effect) (Gottwald *et al.*, 2007; Gottwald *et al.*, 2008; Monteiro *et al.*, 2013; Shen *et al.*, 2013; Sétamou & Bartels, 2015; Tomaseto *et al.*, 2015; Chinelato, 2017). Monteiro *et al*. (2013) and Chinelato (2017) showed that 80% of HLB-eradicated trees werein the first 80-160 m from the border of the orchards. Gasparoto *et al.* (2018) found higher values of HLB incidences and rates of disease annual progress at the citrus blocks closer to the edge than those located around inner sections of the farm. Therefore, for successful HLB management, the removal of symptomatic trees and psyllid control must be accomplished regionally by citrus growers (Bassanezi *et al*., 2013b; Bergamin Filho *et al*., 2016). Further than a regional management performed by neighboring commercial properties, noncommercial citrus trees and orange jasmine (*Murraya* spp.), an ornamental plant frequently used in Brazil and an alternative host for *D. citri* and '*Ca.* Liberibacter spp.', also have to be a target on a program of inoculum sources reduction. These plants are usually present in residences, sidewalks, swamps, woods and cattle fields and do not receive any chemical treatment for vector control as they are often unnoticed as important sources of inoculum.

Many studies have demonstrated the higher occurrence of HLB and *D. citri* on the periphery of the properties. This behavior was associated to external sources of inoculum and HLB primary spread (Bergamin Filho *et al*., 2016). However, no studies have demonstrated the HLB incidences, its temporal progress, and *D. citri* distribution in several orchards maintained under strict HLB management and its relationship with commercial and noncommercial neighboring areas. These are the objectives of the present study. We hypothesize that HLB-infected citrus and orange jasmine plants, maintained without insecticide sprays around citrus properties in SPS, are the sources of

inoculum for the primary spread of HLB in managed areas. By consequence, a more effective HLB management could be achieved with the eradication of those noncommercial sources of inoculum.

4.2 Material andMethods

4.2.1 Area

HLB was studied in a large commercial citrus property (~8.000 ha), that performed strict HLB management, located in the central area of SPS, Brazil (Figure 5). The property was composed of 314 rectangular citrus blocks, which ranged from 0.6 to 41.2 ha (average size of 21.0 ha). First, HLB and *D. citri* were studied in 177 citrus blocks distributed in the property and separated in peripheral (85 blocks) and internal (92 blocks) (Figure 5A). The next approach was to study HLB and *D. citri* in five groups of citrus blocks uniform in age and variety, located at different areas of the property, and also separated in peripheral and internal blocks (Figure 5B and Table 6). Finally, the findings on HLB and *D. citri* distribution in the property were compared with external HLB inoculum sources found by the surveys conducted by employs of the property.

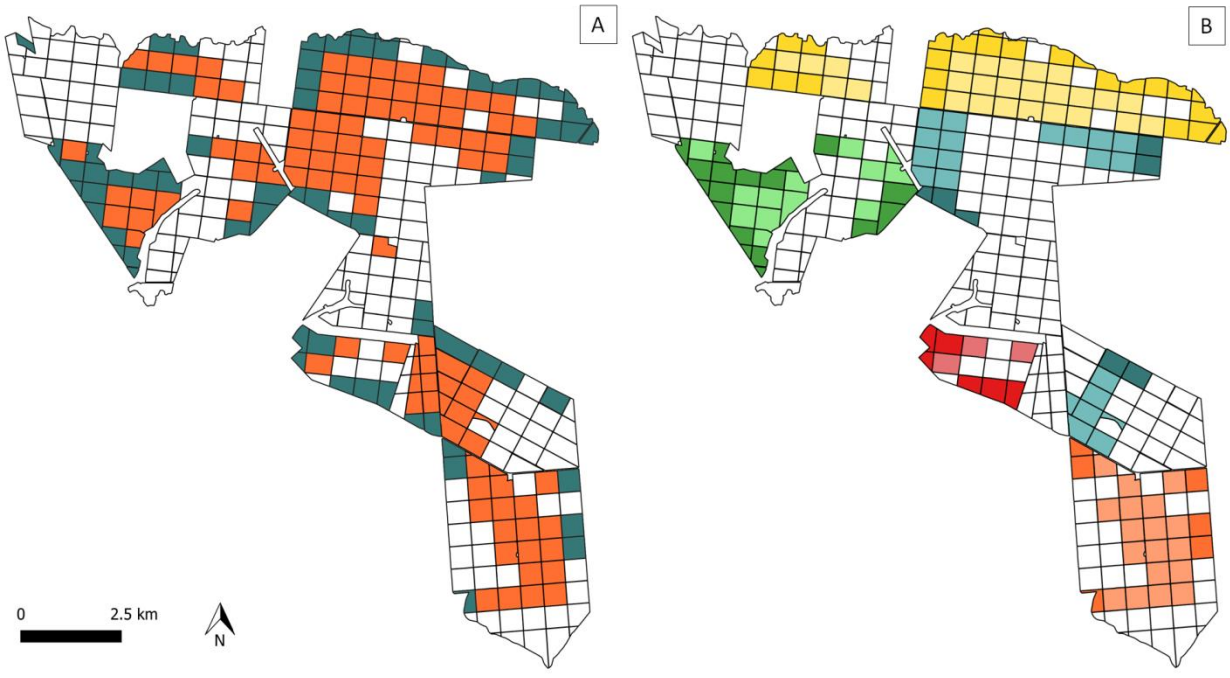

Figure 5. Commercial citrus property composed of 314 citrus blocks of which 177 were separated in peripheral (blue) and internal (orange)(A) and 144 were separated into five groups, uniform in age and varieties: 1985-86 (orange), 1993-94 (yellow), 1997 (green), 2001 (red) and 2009 (blue) (B). Darker and lighter colors represent peripheral and internal blocks, respectively. White square, inside the farm, on northwest side

represent a permanent preservation area. Citrus blocks surrounded by citrus were considered to be internal and citrus blocks with at least one side facing the property external area or the permanent preservation area were considered to be peripheral.

Table 6. Detailed information about the citrus blocks groups 1985-86, 1993-94, 1997, 2001 and 2009. Number of citrus blocks, number of internal and peripheral citrus blocks, varieties of scion and rootstock and total area of each group are presented.

Group	Citrus blocks	Internal/external Citrus blocks	Scion/rootstocks	Area (ha)
1985-86	25	19/6	Natal x Rangpur lime	589.3
1993-94	51	26/25	Valência Argentina x Rangpur lime	1,124.1
1997	31	13/18	Valência Argentina x Rangpur lime	591.3
2001	9	3/6	Natal x Rangpur lime	190.0
2009	28	21/7	Pera Rio x Sunki mandarin (19) Hamlin x CitromeloSwingle (8)	619.0

4.2.2 HLB management

The studied property performed a strict management for HLB, with frequent inspections and eradication of symptomatic trees, monitoring and frequent insecticide sprays for *D. citri* control and the use of healthy plants for new citrus blocks planting or replanting of eradicated trees. From 2014, regional management was carried, by the property's employs, for inoculum sources reduction neighboring the property.

Surveys for HLB symptomatic trees were performed in every tree of each citrus block, at least four times per years, from 2007, by a professional team. From 2012, the surveys of adult blocks (>3 years old) counted with the use of tractor-pulled platforms to assist in the detection of HLB symptom (Belasque *et al.*, 2010). Confirmation of infection was made by PCR analysis when the symptomatology wasn't conclusive. Symptomatic trees, when detected, were eradicated within 30 days.

Monitoring of ACP population was performed by visual inspections of branches end detection in traps. ACP visual inspections were performed at least twice a month, by a professional team, on 1% of all trees, three branches per tree of each block, for detection of eggs, nymphs or adults of *D. citri*. Detections by traps were performed by yellow adhesive traps of 10 x 30 cm (ISCA) fixed in the upper third of trees, placed on the periphery of the citrus blocks, at least one trap per block. Traps

were analyzed weekly, by a professional team, for detection of *D. citri* and evaluations were carried out directly in the field until Sep 2014, and, after, inside property's facilities.

D. citri chemical control was performed with contact insecticides applied via ground, aerial or in border sprays. Ground sprays were carried with tractor and turbo sprayer (1.000 L/ha), at least twice a month. Frequency of sprays followed a risk scale: blocks up to two years received weekly sprays; blocks over two years received applications twice a month when located on the periphery of the property or near forest and marsh; and monthly when located internally. Ground sprays were intensified according to *D. citri* detection. Five to six aerial sprays were performed per years, by airplane (Ipanema, 5.0 L/ha - application range of 18 m), on the entire property based on the Phytosanitary alert recommendation (Fundecitrus, 2018b). Besides contact insecticide sprays, blocks up to two years received two systemic insecticide applications (neonicotinoids - 200 to 1000 mL/tree), via drench, every two months between Sep and Mar (period of higher *D. citri* population).

The regional management was performed, by employs of the property, from 2014 and counted on the execution of inspections on the external area, within a radius of 5 km from the edge of the property. Thus, inspection teams searched for noncommercial citrus or orange jasmine trees in swamps, woods, cattle fields, communities and backyards. When these trees were found, the eradication of the trees or the chemical control of the vector were negotiated with the owner. Release of *Tamarixia radiata* (Waterston, 1922) (Hymenoptera: Eulophidae), a natural enemy of *D. citri*, was also used for regional management.

4.2.3 Data analysis

First, HLB and *D. citri* were studied in 177 citrus blocks distributed in the property. Blocks were composed of different combinations of sweet oranges scions and rootstock and were planted between 1985 and 2009. These blocks were separated, for comparison, in peripheral and internal citrus blocks (85 and 92, respectively). The next approach was to study HLB and *D. citri* in peripheral and internal blocks of each of the five groups of citrus blocks (1985-86, 1993-94, 1997, 2001 and 2009), uniform in age and varieties, and located at different areas of the property. Finally, the findings on HLB and *D. citri* distribution in the property were discussed with external inoculum sources found by the regional management. Data for eradication of symptomatic trees (for 177 blocks), capture of *D. citri/*trap (of 296 blocks) and local, type and amount of external sources of inoculum were provided by the studied property, for this study.

Data of eradication of symptomatic trees (from the first eradication performed in each citrus block until 2014-2016) was used to calculate the cumulative disease proportion and the area under

the disease progress curve (AUDPC), for the 177 blocks. AUDPC was calculated standardized in time (AUDPC*) according to the equation cited by Campbell & Madden (1990). Cumulative annual proportion of HLB-eradicated trees was used to the fitting of the temporal models logistic [y=1/(1+((1/Y_0)-1)*exp(-r*t))] and Gompertz [y=exp(-(-LN(Y_0))exp(-r*t))], by linear regression. For equations, y is the proportion of eradicated trees at the time t, Y_0 is a parameter related to the initial inoculum and r is the estimated disease progress rate. The fitting of the temporal models to cumulative annual proportion of HLB was evaluated by the adjusted coefficient of determination (R^2*) and the distribution of the residues. The number of trees eradicated in the first eradication of each citrus block was considered to be the real initial inoculum (real Y_0), despite the variable incubation period of HLB.

Data of capture of *D. citri/*trap (from Jun 2012 until Dec2016), for the 296 citrus blocks, was used to calculate the average *D. citri/*trap of each block (considering four traps evaluation per month) and the population dynamics of the vector. The HLB incidence, AUDPC*, rate of disease progress (r), real Y_0 and average *D. citri/*trap were compared, by the non-parametric test of Kruskal-Wallis, between the peripheral and internal blocks of the 177 citrus blocks and the five uniform groups (1985-86, 1993-94, 1997, 2001 and 2009). These same variables plus the disease incidence estimated by the temporal model were correlated, by the non-parametric Spearman's rank-order correlation, between the 177 citrus blocks. The distribution of the variables HLB incidence, AUDPC*, rate of disease progress (r) and average *D. citri*/trap in the property, and the external inoculum sources of HLB found on a 5 km radius regional management, were presented in QGIS maps (Graduated style).

4.3 Results

Temporal analyses indicated that the Gompertz and logistic models fitted well to HLB annual incidence for the epidemic occurring under intense management in central SPS. Both models fitted to the 177 citrus blocks studied (p≤0.05). Average adjusted R^2 (R^2*) was 0.95 (ranging from 0.78 to 0.99) and 0.91 (ranging from 0.62 to 0.99) for the Gompertz and logistic models, respectively. Besides presenting R^2* generally superior, Gompertz model also presented better distribution of the residues and was chosen as the best temporal model to describe HLB epidemic in the conditions this study was performed. Gompertz model, fitted to HLB annual progress of 8 citrus blocks, and the respective rate of disease progress (rG) and R^2* are presented in Figure 6.

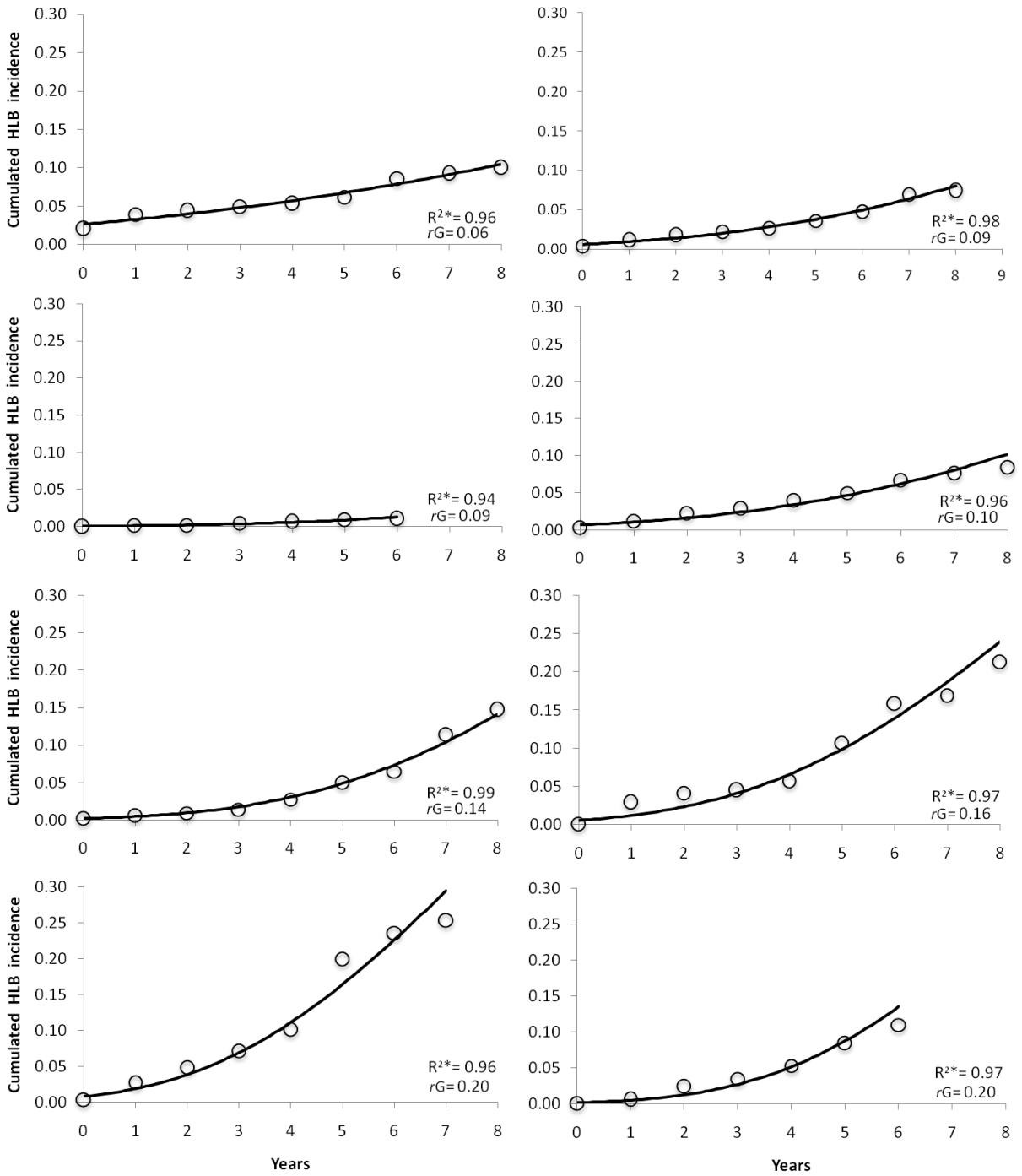

Figure 6. The Gompertz temporal model (black line) fitted to HLB annual proportion (circles) in 8 of the 177 citrus blocks tested. Respective rates of disease progress (*r*G) and adjusted R^2 (R^2*) are presented.

HLB Incidence, AUDPC*, *r*G and average *D. citri*/trap differed ($p \leq 0.05$), for the 177 citrus blocks, between blocks located at the periphery or internally at the property (Table 7). However, the difference wasn't so great for *r*G (0.125 vs. 0.139, p=0.04) and there wasn't difference for real Y_0 between the location of the citrus blocks in the property. The slopes of the regression lines, i.e., the *r*G, for the 177 citrus blocks, presented an average of 0.13 (ranging from 0.04 to 0.27) and 98 citrus blocks (55%) presented *r*G ≤0.13 (of which 59 were intern and 39 were peripheral). For the logistic

temporal model, the average rate was 0.52 (ranging from 0.14 to 1.22). Average HLB Incidence, AUDPC*, real Y_0 and *D. citri*/trap, for the 177 citrus blocks, was 0.114, 0.046, 0.005 and 0.016, respectively.

Table 7. Average, median, minimum (Min) and maximum (Max) HLB incidence, AUDPC*, rate of disease progress (*r*G), real Y_0 and average *D. citri*/trap, of the 177 citrus blocks separated in peripheral and internal blocks. *p*-values resulted for the comparison between intern and peripheral blocks are presented.

	Intern				**Peripheral**				
	Average	Median	Min	Max	Average	Median	Min	Max	*p*-value
Incidence[1]	0.094	0.081	0.005	0.277	0.135	0.111	0.011	0.463	0.0004
AUDPC*[1]	0.042	0.032	0.003	0.127	0.051	0.047	0.003	0.137	0.0072
***r*G**	0.125	0.124	0.042	0.223	0.139	0.139	0.059	0.267	0.0446
Real Y_0	0.005	0.002	<0.001	0.042	0.005	0.003	<0.001	0.046	0.1294
***D.* citri/trap**[2]	0.008	0.004	0.000	0.054	0.025	0.021	0.000	0.096	<0.0001

[1]Cumulative HLB incidence and AUDPC* data from citrus blocks since the first eradication of HLB-symptomatic trees until 2014-16
[2]*D. citri* data from Jun 2012 until 2014-16

Maps of the distribution of HLB incidence, AUDPC*, *r*G (for 177 citrus blocks) and *D. citri*/trap (for 314 citrus blocks) in the property are presented in Figure 7. HLB incidence and AUDPC* presented a very similar spatial distribution. Of the 25 highest incidences observed in the 177 blocks (>0.21 proportion of HLB-infected trees/block), 18 were located on the periphery of the property. For the average of *D. citri*/trap, 29 citrus blocks presented no detection on the period studied, of which 3 were peripheral and 26 were internal. The distribution of the variables (Figure 7) demonstrated that the higher values of HLB incidence, AUDPC*, *r*G and *D. citri*/trap are commonly related to the periphery. However, higher values of the variables can also be seen in some internal blocks and lower values can be seen in peripheral blocks.

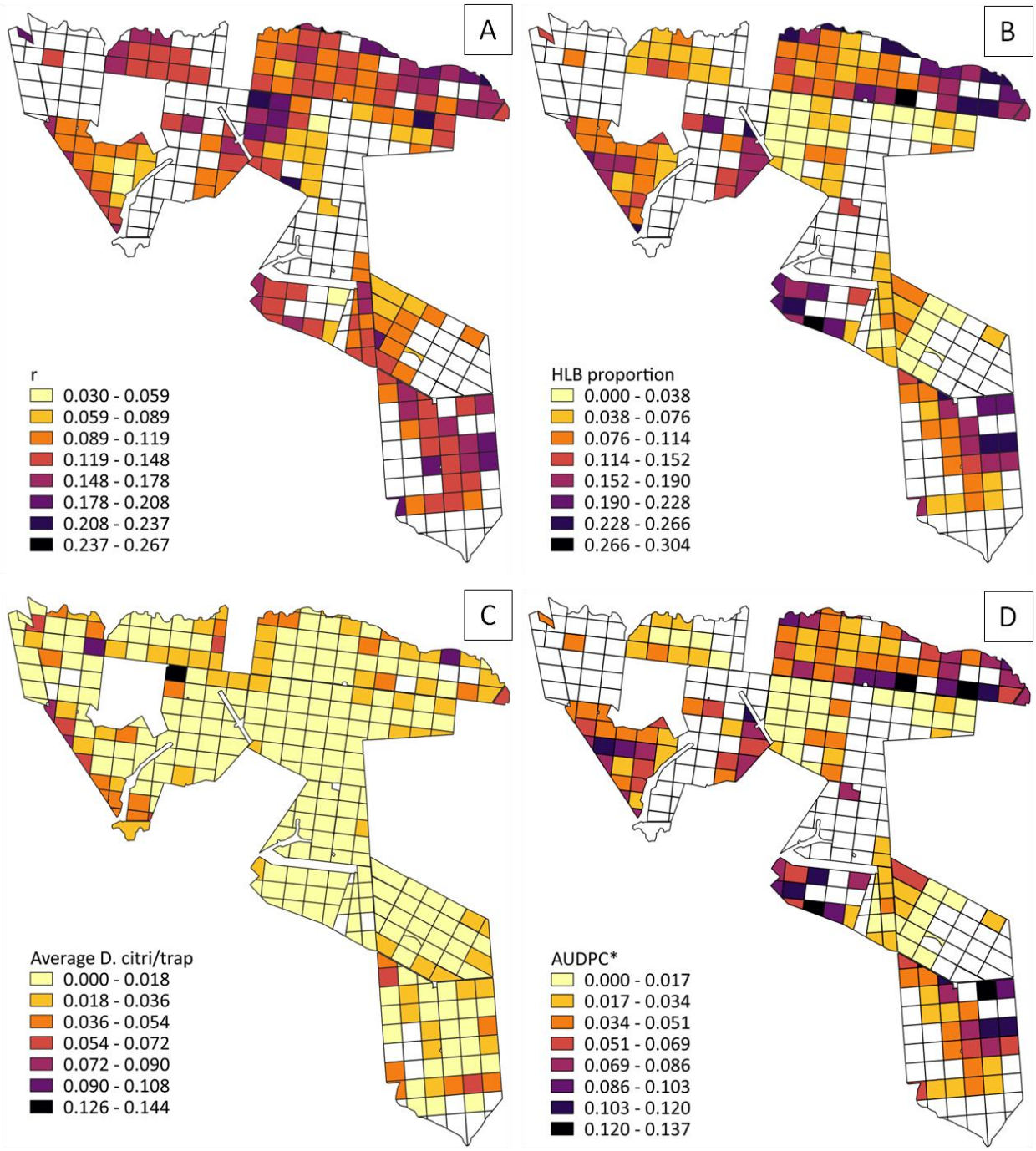

Figure 7. Maps of the distribution of HLB progress rate (obtained by the Gompertz model, *r*) (A), HLB incidence (in proportion) (B), AUDPC* (D) (for 177 citrus blocks) and average *D. citri*/trap (D) (for 296 citrus blocks). HLB incidence and AUDPC* data from citrus blocks since the first eradication of HLB-symptomatic trees until 2014-16. *D. citri*/trap data from Jun 2012 until 2014-16.

Statistically significant ($p \leq 0.05$) Spearman correlations were observed between the variables HLB incidence, real Y_0, HLB incidence estimated by the Gompertz model (Incidence G), rate of disease progress (*r*G), AUDPC* and average *D. citri*/trap, except between real Y_0 and average *D. citri*/trap (Table 8). The strongest correlations (>0.95) were observed between HLB incidence, HLB incidence G

and AUDPC*. In general, the variable that presented the weakest correlations was the average *D. citri*/trap.

Table 8. Spearman correlations of HLB incidence, real Y_0, HLB incidence estimated by the Gompertz model (Incidence G), rate of disease progress (*r*G), AUDPC* and average *D. citri*/trap, for the 177 citrus blocks.

	Real Y_0	**Incidence G**	***r*G**	**AUDPC*[1]**	**Average *D. citri*/trap[2]**
Incidence[1]	0.59	0.98	0.36	0.95	0.30
Real Y_0		0.58	−0.39	0.74	0.09[ns]
Incidence G			0.39	0.96	0.31
***r*G**				0.19	0.21
AUDPC*[1]					0.25

[ns] Variables with no correlation (p>0.05)
[1]HLB incidence and AUDPC* data from citrus blocks since the first eradication of HLB-symptomatic trees until 2014-16
[2]*D. citri*/trap data from Jun 2012 until 2014-16

Average, minimum and maximum HLB incidence, AUDPC*, *r*G, real Y_0, and average *D. citri*/trap for the five uniform groups located at different areas of the property are presented in Table 9. Groups 1985-86, 1993-94, 1997 and 2001 presented higher averages of HLB Incidence, AUDPC* and real Y_0 than the group 2009. On the other hand, average *r*G was very similar between the five groups (ranging between 0.12 and 0.14). For *D. citri*/trap, it was observed smaller averages of vector detected per trap on groups 2001 and 2009 rather than the older groups. For groups 1985-86, 1993-94, 1997, 2001 and 2009 it was observed 2, 11, 5, 2 and 5 blocks with no *D. citri* detection on the period studied. The difference between the cumulative incidences of the groups can be more clearly seen in Figure 8. Groups 1985-86, 1993-94, 1997, 2001 showed an increase in the incidence of HLB from 2011-12.

Table 9. Average, minimum (Min) and maximum (Max) HLB incidence, AUDPC*, *r*G, real Y_0 and average *D. citri*/trap of the five uniform groups (1985-86, 1993-94, 1997, 2001 and 2009).

Group	Incidence[1] (Min - Max)	AUDPC*[1] (Min - Max)	*r*G (Min - Max)	Real Y_0 (Min - Max)	*D. citri*[2] (Min - Max)
1985-86	0.137 (0.051 - 0.253)	0.061 (0.023 - 0.121)	0.143 (0.102 - 0.205)	0.005 (<0.001 - 0.033)	0.023 (0.000 - 0.058)
1993-94	0.137 (0.041 - 0.277)	0.052 (0.015 - 0.127)	0.141 (0.087 - 0.232)	0.005 (<0.001 - 0.046)	0.019 (0.000 - 0.096)
1997	0.135 (0.046 - 0.266)	0.058 (0.022 - 0.111)	0.116 (0.057 - 0.172)	0.007 (0.001 - 0.022)	0.024 (0.000 - 0.085)
2001	0.195 (0.057 - 0.304)	0.088 (0.030 - 0.137)	0.126 (0.053 - 0.166)	0.013 (0.002 - 0.036)	0.006 (0.000 - 0.021)
2009	0.026 (0.005 - 0.073)	0.010 (0.003 - 0.020)	0.136 (0.074 - 0.234)	0.001 (<0.001- 0.005)	0.011 (0.000 - 0.029)

[1]HLB incidence and AUDPC* data from 2007 to 2014 for group 1985-86, from 2007 to 2015 for groups 1993-94, 1997 and 2001 and from 2011 to 2016 for group 2009
[2]*D.* citri/trap data from Jun 2012 to Jun 2015 for group 1985-86 and from Jun 2012to Dec 2016 for groups 1993-94, 1997, 2001 and 2009

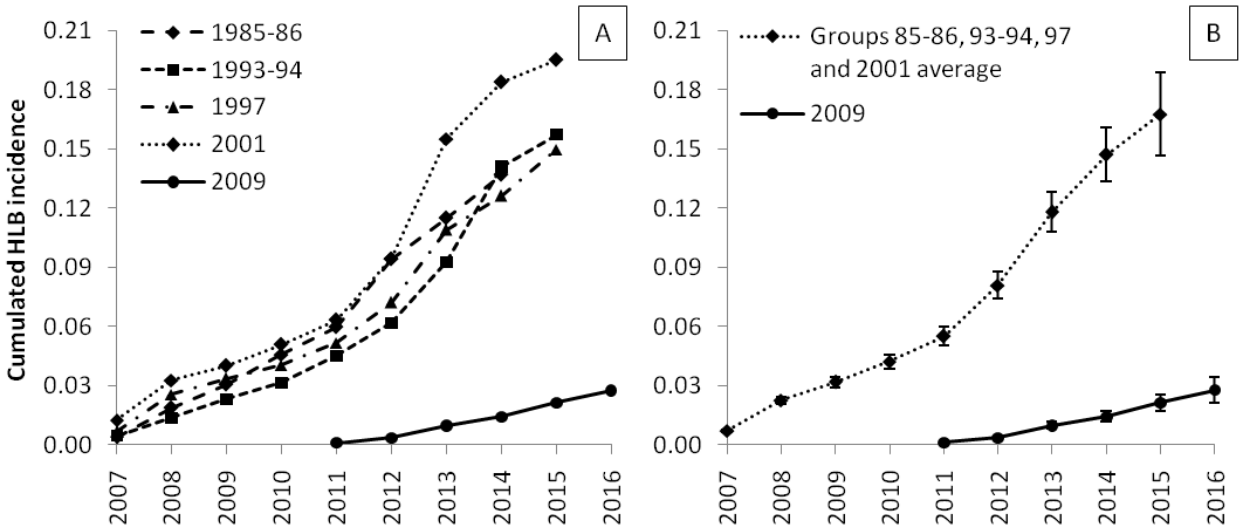

Figure 8. Cumulative HLB incidence from 2007 to 2014 for group 1985-86, from 2007 to 2015 for groups 1993-94, 1997 and 2001 and from 2011 to 2016 for group 2007 (A). Average cumulative HLB incidence of the oldest groups (1985-86, 1993-94, 1997 and 2001) and group 2009 (B).

The population dynamics of the vector in the property versus each of the five groups is presented in Figure 9. Although the group 2001 only showed detection after the second quarter of 2016, the groups 1985-86, 1993-94, 1997 and 2001 presented peaks of *D. citri*/trap higher than the mean average *D. citri*/trap of the entire property, different from group 2009. Generally, the *D. citri*/trap peaks were related to the period between Jul and Jan.

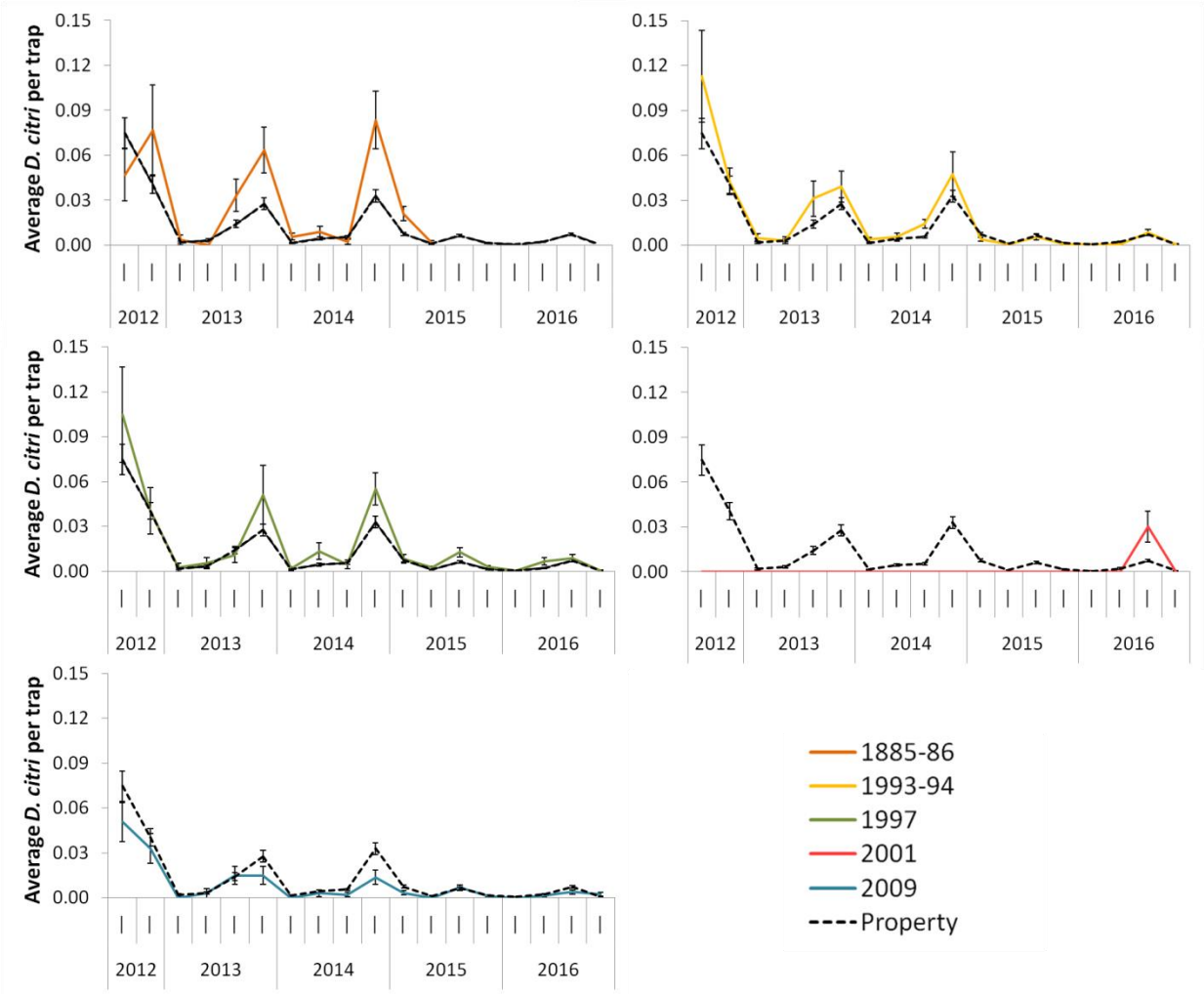

Figure 9. *D. citri* population dynamicsof the property (black dashed line) versus each of the five groups. Group 1985-86 between Jun 2012 and Jun 2015 and groups 1993-94, 1997, 2001 and 2009 between Jun 2012 and Dec 2016.

HLB Incidence, AUDPC*, *r*G, real Y_0 and average *D. citri*/trap were compared, for each of the five groups, between peripheral and intern citrus blocks (Table 10). For group 1985-86, the only difference ($p \leq 0.01$) was observed on the average of *D. citri*/trap, which was higher on peripheral blocks. The group 1993-94 presented higher ($p \leq 0.01$) HLB incidence, *r*G and average *D. citri*/trap on peripheral blocks. The group 1997 presented higher ($p \leq 0.05$) *r*G and average of *D. citri*/trap on peripheral blocks. The groups 2001 and 2009 didn't differ ($p > 0.05$) in any of the variables compared between peripheral and internal citrus blocks.

Table 10. Average, minimum (Min) and maximum (Max) HLB incidence, AUDPC*, *r*G, real Y_0 and average *D. citri*/trap of peripheral and internal citrus blocks of the groups 1985-86, 1993-94, 1997, 2001 and 2009. *p*-values resulted for the comparison between intern and peripheral blocks are presented.

	Incidence[1]	AUDPC*[1]	*r*G	Real Y_0	Average *D. citri*/trap[2]
			85-86		
Periphery	0.166	0.072	0.164	0.004	0.040
Intern	0.128	0.057	0.136	0.005	0.017
***p*-value**	0.112	0.203	0.112	0.703	0.007
			93-94		
Periphery	0.163	0.056	0.155	0.005	0.029
Intern	0.113	0.048	0.126	0.004	0.010
***p*-value**	0.014	0.127	<0.001	0.888	<0.001
			1997		
Periphery	0.143	0.059	0.126	0.005	0.037
Intern	0.124	0.058	0.100	0.010	0.007
***p*-value**	0.317	0.749	0.028	0.215	<0.001
			2001		
Periphery	0.197	0.082	0.138	0.008	0.008
Intern	0.191	0.100	0.101	0.022	0.003
***p*-value**	0.796	0.302	0.070	0.071	0.221
			2009		
Periphery	0.029	0.012	0.137	0.001	0.015
Intern	0.025	0.010	0.136	0.001	0.010
***p*-value**	0.212	0.326	0.771	0.770	0.209

[1]HLB incidence and AUDPC* data from 2007 to 2014 for group 1985-86, from 2007 to 2015 for groups 1993-94, 1997 and 2001 and from 2011 to 2016 for group 2009
[2]*D.* citri/trap data from Jun 2012 to Jun 2015 for group 1985-86 and from Jun 2012 to Dec 2016 for groups 1993-94, 1997, 2001 and 2009

Many potential external sources of inoculum, composed mainly by noncommercial citrus or orange jasmine trees, were found around the property (Figure 10 – green and yellow circles). These trees were distributed mainly to the north and west sides of the property. The largest quantities of external inoculum sources found in a single site (yellow circles) were located: in the northwestern area (totaling 3,050 trees) near the group 1997; in the northeast area (totaling 439 trees), near the group 1993-94; and in the southeast area of the property (totaling 7,544 trees), near the group 1985-86. Commercial properties that performed HLB management (trees eradication and insecticide sprays) were not considered to be external inoculum sources. Also, in addition to external sources of inoculum, many noncommercial and HLB-symptomatic trees or shoots were found within the property (red circles). These plants are mostly shoots in citrus blocks that were eradicated for reform and trees that grew in woods and swamps within the property. The internal sources of inoculum were mainly related to groups 1985-86, 1997 and 2001.

Higher HLB incidence and average *D. citri*/trap (Figure 10B and C) occurred near external (or non-external) sources of inoculum, as can be seen for the northwestern, northeast and southeast areas of the property. These areas presented the largest quantities of external inoculum sources found in a single site (yellow circles). However, in the central area of the property (the area composed by the group 2001), very high HLB incidences as well as external and internal sources of inoculum were found, but low *D. citri*/trap averages were observed.

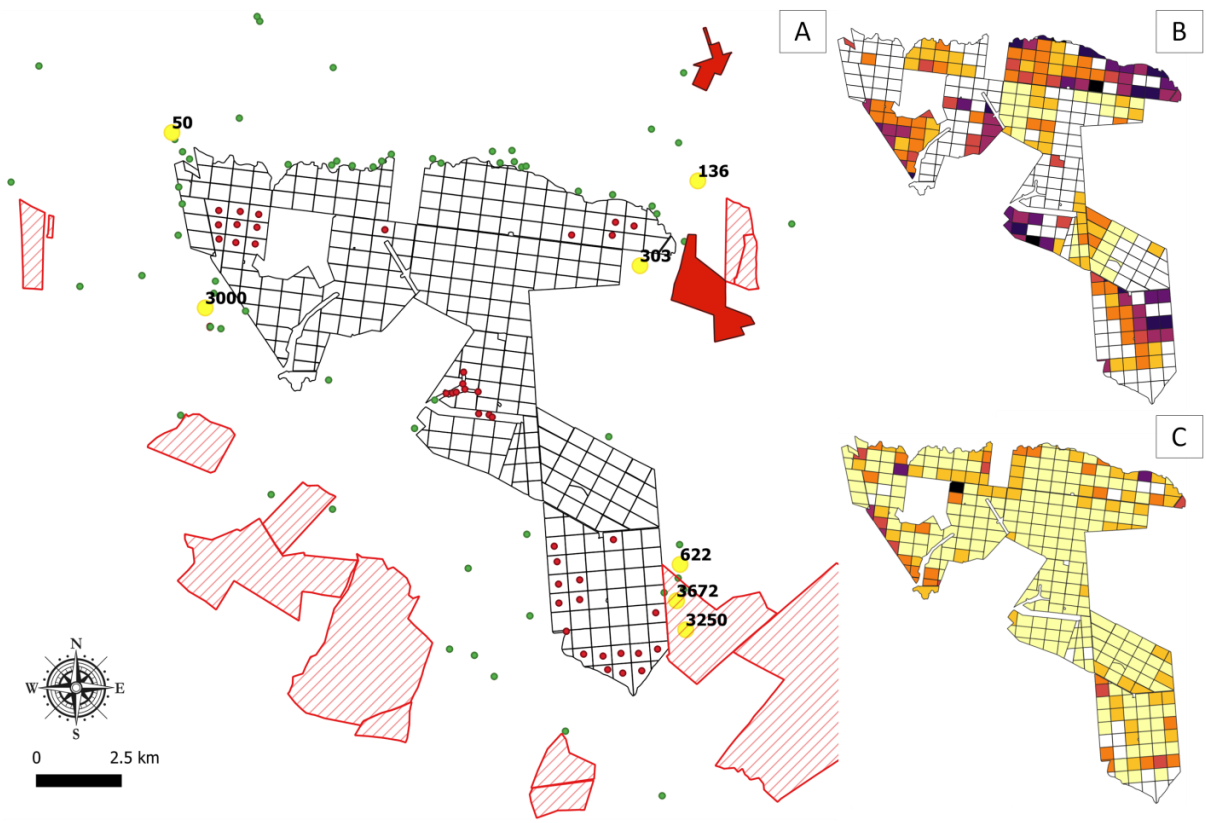

Figure 10. External potential sources of HLB inoculum found around the property (A). Areas striped in red are neighboring commercial properties that performed HLB management (trees eradication and insecticide sprays). Fully red areas are commercial citrus properties that didn't manage HLB. Red circles are HLB-symptomatic trees or shoots (1 to 5 shoots) found inside the property in swamps or blocks eradicated for renovation. Green circles are HLB-symptomatic citrus trees or orange jasmine (1 to 45 trees) found outside the property in swamps, woods, cattle fields and backyards. Yellow circles (and numbers) represent the biggest external inoculum sources found around the property. Maps of the distribution of HLB incidence (B) for 177 citrus blocks and average *D. citri*/trap (C) for 296 citrus blocks are presented.

4.4 Discussion

Temporal analyses indicated that both the Gompertz and logistic temporal models fitted well to HLB annual incidences for the epidemic occurring under intense management in central SPS. According to Bergamin Filho *et al.* (2008), the fitting of both models to HLB disease progress is

related to the lack of asymptotic data, i.e., data in which disease incidence approaches the highest level. This fact, in turn, is related to the impossibility of maintaining orchards with high incidence of the disease, since they will be economically unviable long before the incidence reaches 100%. Due presenting better distribution of residues and higher R^2* than the logistic, Gompertz was chosen as the temporal model that best described the HLB epidemic studied.

Even HLB being a multiyear disease (polyetic), given the perennial nature of citrus and comparing the epidemic with other arboreal diseases, HLB is considered to increase rapidly (Gottwald, 2010a). The rapid rate of HLB increase may be associated with the presence of large amounts of inoculum and high population of the vector. Characteristics of the HLB, such as the short latent period and the long incubation period difficult the identification of inoculum sources and disease control. Furthermore, the rapid distribution of the pathogen in the plant, although not regular, may also be associated with the fast rates of disease progression.

However, the HLB progress rates estimated by the Gompertz (*r*G= 0.04 to 0.28) and logistic (*r*L= 0.14 to 1.22) models here presented were lower than most of the progress rates for HLB published in literature. Rates of the annual progress of a HLB epidemic, studied in a large area in south Florida - USA, estimated by the Gompertz model, ranged from 0.44 to 0.97, while logistic rates varied from 0.72 to 2.73 (Gottwald *et al.*, 2010b). In HLB epidemics studied in Brazil, in 2013, under conditions of strict management, logistic rates varied from 0.82 to 2.77 (Bassanezi *et al.*, 2013a,b). Recently, also in Brazil, HLB annual progress rates estimated by the Gompertz model ranged from 0.10 to 0.40 for 85% of citrus blocks in conditions similar to those of this study, with strict disease management in a large property (Gasparoto *et al*., 2018). However, even in this last case in which the conditions were similar, only a few rates were smaller than *r*G= 0.13, as the 98 blocks (55% of 177 blocks) of this study. Temporal epidemiological parameters of HLB, such as *r*G, are dependent on inoculum sources, vector populations, age of the orchard and numerous environmental factors (Bassanezi & Gottwald, 2009; Gottwald, 2010a) and may, together with the disease management differences, explain the variation of HLB progress rates.

Higher HLB incidences and annual disease progress rates were observed at peripheral citrus blocks by Gasparoto *et al*. (2018). Those and other previous authors related this to the vector migration from external sources of inoculum and emphasized the need for stricter disease control at these areas as well the need to extend the control to external sources of inoculum. When *r*G, HLB Incidence, initial inoculum (real Y_0), AUDPC* and average *D. citri*/trap, of the 177 citrus blocks, were compared between citrus blocks located at the periphery or internally, higher values were found in blocks located at the periphery of the property, except for real Y_0. Even *r*G being little variable, it was higher in peripheral (0.139) than in internal (0.125) citrus blocks. These results were consistent with

the maps of distribution of HLB incidence, AUDPC* and *D. citri*/trap, which were more clearly associated with peripheral blocks than *r*G.

Peripheral and internal citrus blocks didn't differ in the variable real Y_0. Model fitting of mathematical functions, such as the Gompertz and logistic, to temporal data of epidemics, is highly dependent of the real Y_0. Small variations at the parameter Y_0 can result in high variations at the rate of disease progress (*r*). As presented in Table 8, real Y_0 correlated negatively with *r*G (Spearman= −0.39, *p*-value= 0.05). By consequence, HLB epidemics showing similar temporal rates (*r*) can differ about the real Y_0, HLB final incidence and AUDPC*. For example, the citrus blocks that presented the highest incidences of removed trees in our study, weren't, necessarily, those with the highest *r*G or *r*L, the opposite being also true. The last two graphs of Figure 6 presented *r*G= 0.20, but HLB incidences were 0.25 and 0.10, reached in 7 years (between 2007 and 2014). Both blocks were planted in 1985, but varied in real Y_0 and location, which were 0.0036 (peripheral, 0.25 HLB incidence) and 0.0003 (internal, 0.10 HLB incidence). As presented at Table 8, HLB incidences, real Y_0 and AUDPC* presented higher correlation between each other than with *r*G. These results indicate that HLB epidemics cannot be compared only by temporal progress models. HLB final incidences and AUDPC*, as observed here, can be more useful to compare HLB-affected areas than only annual progress rates adjusted by temporal models. The calculation of the AUDPC* allowed a more adequate comparison of HLB incidences between blocks with different disease evaluation period. However, even with incidences of HLB having been evaluated in different periods for each block, HLB Incidence and AUDPC* presented a very similar spatial distribution at the property and a high Spearman correlation (0.95).

The average of *D. citri*, for the 177 citrus blocks, was 0.016 *D. citri*/trap (the same average of 0.016 was observed for the 296 citrus blocks) and it's a very low average compared to the presented in the literature. Even the average *D. citri*/trap observed in peripheral blocks is considered low (0.025). In Florida, mean number of 6 and 10 *D. citri*/trap were reported by Hall *et al.* (2008). In Brazil, Bassanezi *et al.*(2013b) observed 0.08 to 0.46 adults per trap. The strict HLB management and the large size of the property may partially explain the lower *D. citri* population densities in a commercial citrus grove. In relation to the population dynamics of *D. citri*, the vector peaks were observed in the periods between Jul and Jan of the following year. Similar observations were made in SPS by Yamamoto *et al.* (2001), Leal (2009) and Santos (2013). The authors related the peaks with the higher temperatures, air humidity, and intense emission of vegetative flushes in citrus trees at the spring season.

The average *D. citri*/trap was the variable that differentiated more between internal (0.008) and peripheral (0.025) citrus blocks. This result corroborates with Sétamou & Bartels (2015), who observed a higher population density of the vector in the fields located in the border of the property.

Even so, in our study, some blocks of periphery presented average *D. citri*/trap equal to internal blocks or equal to zero. This difference is related to the predominance of the HLB primary dissemination and to the long-distance vector dispersion. Thus, peripheral blocks are more subject to external sources of inoculum, despite the strict management adopted in some farms of SPS. This also explains absence of vector detection, in the period studied, for some of the peripheral blocks, since they are at the periphery but might not be close to external sources of inoculum.

In general, the variable average *D. citri*/trap presented the weakest correlations. Other variables, such as HLB incidence, are more influenced by the strict management of the disease, performed in the property. Since the management is not efficient against the migration of infective *D. citri* from external plants and farms, this variable is more affected by the location of the blocks (peripheral or internally) than the local vector control. This explains the complexity of HLB management, compared to other diseases, since the disease can be, as in the case presented here, more dependent of external sources of inoculum.

Higher averages of HLB incidence, AUDPC* and realY_0 were found in groups 1985-86, 1993-94, 1997 and 2001 than in the group 2009 (Table 9 and Figure 8). It was also possible to observe a higher increase in disease progress in the years 2011-12, the same years that platforms for inspection for symptomatic HLB trees were implemented. Platforms allowed a higher HLB-symptomatic tree detection than walking inspections (Belasque *et al.*, 2009). Groups1985-86, 1993-94, 1997 and 2001 also presented peaks of *D. citri*/trap higher than the mean average of the property, different from group 2009. The 2001 group present almost all blocks facing external areas of the property, pattern not existing for 2009 group. In addition, trees at the 2009 group were cultivated in a period with more knowledge generated about HLB and vector spread, especially for vector control.

All groups showed few differences between internal and peripheral blocks. This can be explained by the small variation of the variables within each group. The variable that most differed was average *D. citri*/trap (differed for groups 1985-86, 1993-94 and 1997). However, despite the differences in the other variables, *r*G was very similar (ranging between 0.12 and 0.14) between the five groups. It is interesting to note blocks of trees with different ages, varieties, located in different places in a very large area, presenting very similar disease progress rates. As discussed previously in this study, temporal models as Gompertz and logistic are not adequate to capture the primary spread that governs the epidemics in areas under strict management. Though these models can be used to compare HLB epidemics, some other variables are necessary to a better description of the disease pattern.

The findings on HLB and *D. citri* distribution inside the property were compared with the external HLB inoculum sources found by the surveys conducted by employs of the property (Figure 10). Those surveys started in 2014, but were intensified after that. The objective was to detect and

remove possible sources of inoculum of HLB/vector. A great number of noncommercial external sources of inoculum was found around the property, and seemed to be in accordance to the HLB incidence and average *D. citri*/trap spatial distribution. In addition, many plants that could serve as source of inoculum for HLB were found even within the property. It was not possible to analyze the HLB epidemics of all block of the property. However, the spatial distributions of the average *D. citri*/trap in the blocks and the potential inoculum sources localized inside and outside of the property are very useful to establish the probable relationship between noncommercial plants, i.e., plants not sprayed with insecticides for vector control, and the HLB infections occurred in the studied blocks.

HLB Incidences, average *D. citri*/trap and the *r*G found in this study were low compared to other HLB epidemics described in the literature. This must be related to the intense management of the disease carried out in the studied property and also to the large size of that property. The highest values of HLB incidences and average *D. citri*/trap were found mainly in peripheral blocks, demonstrating, in turn, the influence of external sources of inoculum to the HLB epidemic. In addition, those blocks were in the proximity of sites where most external potential sources of inoculum were found. Thus, yellow stick traps are useful in SPS conditions not only to a better vector control inside of managed areas, but also to identify locations where noncommercial plants could be present around the property.

In the search for ways to reduce the devastating effects caused by HLB in the America's citriculture, many changes are and still will be made to turn the citrus production economically viable. According to da Graça *et al*. (2016), keep an economic production of citrus is the largest challenge ever faced by the citrus industry worldwide. The current scenario involves a lot of resources investment by government and citrus industry in researches focusing in finding and implementing strategies to minimize the infection and the symptoms progress on citrus trees. HLB is a complex disease and the management adopted inside the orchards aren't effective in reducing disease incidences. As justified by Bassanezi *et al*. (2013b), it is necessary to manage the disease regionally. However, as nowadays conducted in important citrus producing areas in America (SPS, other regions in Brazil, Florida, Mexico), the "regional management" adopted by commercial growers corresponds to only insecticide sprays applied in a short period by neighboring farms. As demonstrated here, low levels of vector population and HLB incidences are achievable, in large areas in SPS, when the three practices for HLB management are adopted. In addition, but not less important, as presented here, HLB epidemics occurs by primary spread in managed areas in SPS and noncommercial citrus and orange jasmine plants are the mainly sources of HLB inoculum.

By conclusion, better HLB management could be achieved by the eradication of noncommercial citrus and orange jasmine plants maintained without insecticides sprays for vector

control, together with the three recommended practices for HLB management. Those sources of inoculum are commonly confused with commercial citrus trees maintained treated by insecticides in neighbor areas. However, real attention and efforts have to be used to localize and remove these rutaceous plants, never sprayed for vector control, located inside and outside of commercial properties and until now underestimated as important sources of HLB inoculum.

REFERENCES

Bassanezi RB, Gottwald TR, 2009. Epidemiology of HLB and potencial pathways for introduction. In: *Internacional Worckshop on citrus quarantine pest*.

Bassanezi RB, Belasque J, Montesino LH, 2013a. Frequency of symptomatic trees removal in small citrus blocks on citrus *Huanglongbing epidemics*. *Crop Protection* **52**, 72–77.

Bassanezi RB, Montesino LH, Gimenes-Fernandes N *et al.*, 2013b. Efficacy of area-wide inoculum reduction and vector control on temporal progress of *Huanglongbing* in young sweet orange plantings. *Plant Disease* **97**, 789–796.

Belasque J, Bassanezi RB, Yamamoto PT *et al.*, 2010. Lessons from *Huanglongbing* management in São Paulo State, Brazil. *Journal of Plant Pathology* **92**, 285–302.

Belasque J, Bergamin Filho A, Bassanezi RB *et al.*, 2009. Base científica para a erradicação de plantas sintomáticas e assintomáticas de *Huanglongbing* (HLB, Greening) visando o controle efetivo da doença. *Tropical Plant Pathology* **34**, 137–145.

Bergamin Filho A, Bassanezi RB, Amorim L *et al.*, 2008. Epidemiology of HLB (Huanglongbing). In: *I Taller Internacional sobre Huanglongbing de los cítricos (Candidatus* Liberibacterspp*) y el psílido asiático de los cítricos (Diaphorina citri)*. Hermosillo, Sonora. México, 9.

Bergamin Filho A, Inoue-Nagata AK, Bassanezi RB *et al.*, 2016. The importance of primary inoculum and area-wide disease management to crop health and food security. *Food Security* **8**, 221–238.

Boina DR, Meyer WL, Onagbola EO *et al.*, 2009. Quantifying dispersal of *Diaphorina citri* (Hemiptera: Psyllidae) by immunomarking and potential impact of unmanaged groves on commercial citrus management. *Environmental entomology* **38**, 1250–1258.

Bové JM, 2006. *Huanglongbing*: A destructive, newly-emerging, century-old disease of citrus. *Journal of Plant Pathology* **88**, 7–37.

Bové JM, 2014. *Huanglongbing* or yellow shoot, a disease of Gondwanan origin: Will it destroy citrus worldwide? *Phytoparasitica* **42**, 579–583.

Campbell CL, Madden LV, 1990. *Introduction to plant disease epidemiology*. New York: Wiley.

CDA (Defesa Agropecuaria do Estado de São Paulo), 2017. Compilação dos dados dos relatorios semestrais do greening enviados para a coordenadoria de Defesa Agropecuaria do Estado de São Paulo (CDA). *Dados da Citricultura Paulista*.

Chinelato GA, 2017. Dinâmica temporal e distribuição espacial de *Huanglongbing* (HLB , Greening) e seu inseto vetor (*Diaphorina citri*) Kuwayama (Hemiptera: Liviidae). Dissertação de mestrado: Universidade de São Paulo Escola Superior de Agricultura “ Luiz de Queiroz ”.

Coletta Filho HD, Targon MLPN, Takita MA *et al.*, 2004. First report of the causal agent of

Huanglongbing ('*Candidatus* Liberibacter asiaticus') in Brazil. *Plant Disease* **88**, 1382.

Fundecitrus, 2018a. Levantamento da incidência das doenças dos citros: Greening, CVC e Cancro cítrico no cinturão citrícola de São Paulo e triângulo/sudoeste mineiro. *Fundo de Defesa da Citricultura – FUNDECITRUS*.
Available in: https://www.fundecitrus.com.br/levantamentos/greening

Fundecitrus, 2018b. Phytosanitary Warning System. *Fundo de Defesa da Citricultura – FUNDECITRUS*. Available in: https://www.fundecitrus.com.br/alerta-fitossanitario

Gasparoto MCG, Hau B, Bassanezi RB *et al.*, 2018. Spatiotemporal dynamics of citrus *Huanglongbing* spread: a case study. *Plant Pathology* **67**, 1621–1628.

Gottwald TR, Irey M, Bergamin Filho A *et al.*, 2008. A stochastic spatiotemporal analysis of the contribution of primary versus secondary spread of HLB. In: *Proc. International Research Conference on Huanglongbing*. 247–252.

Gottwald TR, 2010a. Companion Cropping to Manage Parasitic Plants. *Annual review of phytopathology* **48**, 161–177.

Gottwald TR, 2010b. Current epidemiological understanding on citrus *Huanglongbing*. *Annual review of phytopathology* **48**, 119–139.

Gottwald TR, da Graça JV, Bassanezi RB, 2007. Citrus *Huanglongbing*: The pathogen and its impact plant health progress plant health progress. *Plant Health Progress* **36**.

Gottwald TR, Irey M, Gast T *et al.*, 2010. Spatio-temporal analysis of an HLB epidemic in Florida and implications for spread. *Proceedings of the 17 th Conference of International Organization of Citrus Virologists, IOCV*, 84–97.

da Graça JV, Douhan GW, Halbert SE *et al.*, 2016. *Huanglongbing*: An overview of a complex pathosystem ravaging the world's citrus. *Journal of Integrative Plant Biology* **58**, 373–387.

Hall DG, Hentz MG, Adair R, 2008. Population ecology and phenology of *Diaphorina citri* (Hemiptera: Psyllidae) in two Florida citrus groves. *Environmental Entomology* **37**, 914–924.

Leal RM, 2009. Distribuição espacial de *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) e sua relação com a expansão do greening em laranjeira Valência, utilizando geoestatística. Tese: UNESP.

Lewis-Rosenblum H, Martini X, Tiwari S *et al.*, 2015. Seasonal movement patterns and long-range dispersal of Asian citrus psyllid in Florida citrus. *Journal of Economic Entomology* **108**, 3–10.

Martini X, Addison T, Fleming B *et al.*, 2013. Occurrence of *Diaphorina citri* (Hemiptera: Liviidae) in an unexpected ecosystem: The lake Kissimmee State Park Forest, Florida. *Florida Entomologist* **96**, 658–660.

Martini X, Hoyte A, Stelinski LL, 2014. Abdominal color of the Asian citrus psyllid (Hemiptera: Liviidae) is associated with flight capabilities. *Annals of the Entomological Society of America* **107**, 842–847.

Monteiro AB, Pereira De M, Coorientador M *et al.*, 2013. Efeito de um pomar sem manejo de *Huanglongbing* sobre a ocorrência de *Diaphorina citri* e incidência de plantas doentes em uma área vizinha com manejo da doença. Dissertação: Fundecitrus.

Santos TR dos, 2013. Flutuação populacional e distribuição espacial de *Diaphorina citri* kuwayama (Hemiptera: Liviidae) em propriedades citrícolas com manejo intensivo do *Huanglongbing*. Tese: UNESP.

Sétamou M, Bartels DW, 2015. Living on the edges: Spatial niche occupation of asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), in citrus groves. *Plos One* **10**.

Shen W, Halbert SE, Dickstein E *et al.*, 2013. Occurrence and in-grove distribution of citrus *Huanglongbing* in north central Florida. *Journal of Plant Pathology* **95**, 361–371.

Teixeira DDC, Danet JL, Eveillard S *et al.*, 2005. Citrus *Huanglongbing* in São Paulo State, Brazil: PCR detection of the '*Candidatus* Liberibacter' species associated with the disease. *Molecular and Cellular Probes* **19**, 173–179.

Tiwari S, Lewis-Rosenblum H, Pelz-Stelinski K *et al.*, 2010. Incidence of '*Candidatus* Liberibacter asiaticus' infection in abandoned citrus occurring in proximity to commercially managed groves. *Journal of economic entomology* **103**, 1972–1978.

Tomaseto AF, Krugner R, Lopes JRS *et al.*, 2015. Effect of plant barriers and citrus leaf age on dispersal of *Diaphorina citri* (Hemiptera: Liviidae). *Journal of Applied Entomology* **140**, 12.

Yamamoto PT, Paiva PEB, Gravena S, 2001. Flutuação populacional de *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) em pomares de citros na região Norte do Estado de São Paulo. *Neotropical Entomology* **30**, 165–170.